SYNTHESIS OF SOME DAUNOMYCIN-TYPE ANTI-TUMOR ANTHRACYCLINES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITY

HANS DE BIE

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Joannes Franciscus Martinus de Bie

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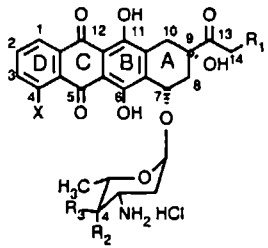
CHAPTER 1

INTRODUCTION.

1.1 General Introduction

The anthracyclines constitute a group of natural antibiotics isolated from cultures of various Streptomyces. Of these, daunomycin (1) and adriamycin (2, Figure 1.1) have been very well investigated over the past 30 years because of their high therapeutic value in cancer chemotherapy[1a-d]. Adriamycin[2] (doxorubicin[3], 2), the most prominent representative of these anti-tumor antibiotics, has been in clinical use since 1974. After this time, it is still one of the most widely used anti-tumor compounds, as it has the widest spectrum of anti-tumor activity and it can be used with a high degree of efficacy against many human cancers. The major side effect which restrict its use is dose-related cardiotoxicity[1,2,4]. This has stimulated intensive research into the synthesis of analogs of adriamycin that are less cardiotoxic. Other goals for synthetic chemists in this area have been to prepare analogs that have greater anti-tumor activity, especially for human cancers that are insensitive to adriamycin, or analogs that might be administered orally. A more recent goal is to find analogs that are able to overcome the phenomenon of multi-drug resistance in cancer cells[5a,b].

Figure 1 1



1	X = OCH_3	R_1 = H	R_2 = OH	R_3 = H	(daunomycin)
2	X = OCH_3	R_1 = OH	R_2 = OH	R_3 = H	(adnamycin)
3	X = OH	R_1 = H	R_2 = OH	R_3 = H	(carminomycin)
4	X= H	R_1 = H	R_2 = OH	R_3 = H	(idarubicin)
5	X = OCH_3	R_1 = OH	R_2 = H	R_3 = OH	(4'-epirubicin)

1.2 History of discovery and evaluation of anti-tumor anthracyclines.

A start for the search of bacterial antibiotics was made by Waksman *et al.*[6] in 1942 when they reported the isolation of the antibiotic actinomycin from a strain of *Streptomyces sp.* In the mid-1950s, Farmitalia in Italy, initiated a program to study anti-tumor compounds produced by novel strains of microbes isolated from soil. In 1957, they isolated a colony of *Streptomyces peucetius* which produced a red pigment. This compound was called daunomycin (1, Figure 1.1) and in 1963, Di Marco *et al.*[7] demonstrated the anti-tumor activity of this new compound. At about the same time, a compound called rubidomycin was isolated by Dubost *et al.*[8] of Rhone

Poulenc (France). Daunomycin and rubidomycin were later found to be the same substance and the combined name of daunorubicin was agreed. The name daunomycin is still used in organic chemistry and this name will be maintained in this thesis.

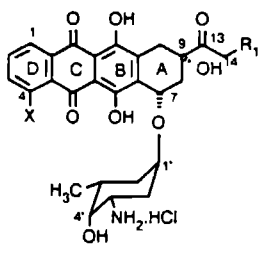
In 1969, Arcamone *et al.*[9] reported the isolation of adriamycin[3] (doxorubicin, 2) from a variant strain of *Streptomyces peucetius* named *caesius*. The compound was quickly introduced into clinical trials. In 1974, adriamycin was approved for marketing in the United States. In the early seventies, a third related compound with an hydroxy group instead of a methoxy group at the C_4 position (3) was isolated by a Russian group[10] from *Actionomadura carminate*. This compound (carmynomycin) is no longer in clinical trials since its anti-tumor activity is inferior to that of adriamycin.

To date, more than 2,000 analogs of adriamycin have been prepared, most of them by semi-synthesis, and they have been evaluated for anti-tumor efficacy. No analog has yet been found which is superior to adriamycin. Apart from daunomycin and adriamycin, two other derivatives are being used world-wide, these are idarubicin (4-demethoxydaunomycin; 4) and 4'-epirubicin (4'-epiadriamycin; 5).

1.3 Mechanism of action and SAR studies.

Since the discovery of the anti-tumor anthracyclines, a large amount of work has been expended in the elucidation of their mode of action. The generally accepted view is that the anthracyclines exert several parallel cytotoxic mechanisms of anti-tumor activity, including intercalation between DNA bases, covalent binding of reactive metabolites to DNA, free radical formation, interaction with the cell membrane and topoisomerase-II-mediated DNA strand breaks[1,2,4,5]. The cardiotoxic effect is mainly ascribed to free radical formation[5a].
The main modifications in the structures of adriamycin and daunomycin which have been evaluated in order to find compounds with a higher therapeutic index or to get more insight in the mechanism of action are shown in Figure 1.2.

Figure 1.2



6	C_{13}=O	⇒	CH_2, CHOH, C=N-R.
7	R_1	⇒	H, OR, SR, OCOR.
8	H-C_4'-OH	⇒	C=O, H-C-I, HO-C_4'-H (epi-diastereomer)
9	NH_2.HCl	⇒	NHCOR, 
10	X	⇒	OCH_3, OH, H.

Anthracyclines of the daunomycin type are composed of an aglycone moiety (ABCD rings) which is named anthracyclinone, and a sugar component, the amino sugar, daunosamine. The aglycone has two chiral centers at C_7 and C_9 in the A ring with the S,S configuration, and the sugar has four chiral centers with the configuration of a L-lyxo-hexose. For anti-tumor activity, the S,S configuration is essential. The majority of the new analogs have been prepared by semi-synthesis, starting with daunomycin or adriamycin, by transformation of the C_{13}-carbonyl group (6), substituent R_1 (7), a carbonyl group in the C ring or by modifications in the sugar part (8,9). Modifications in the D (10) or B rings of the aglycone have been less studied, as these can only be achieved by total synthesis. The most promising derivative that has been discovered so far is idarubicin (4-demethoxydaunomycin, 4). It is used world-wide against acute leukemias but was not able to replace adriamycin against other tumors. Most efforts at modification of the sugar part of the molecule have been concentrated on modification of the 4' position or the amino group. This has resulted in the compound 4'-epirubicin (4'-epiadriamycin, 5), having a 4'-equatorial hydroxy group. The compounds is claimed to have lower cardiotoxicity than adriamycin. Another promising analog has the amino group transformed into a cyanomorpholine group (9). This compound is still under evaluation.

A small number of these derivatives has been prepared by microbial cultures[1d]; a discussion of these particular derivatives is beyond the scope of this thesis.

Relatively few analogs prepared by total synthesis have been evaluated, although total synthesis will certainly allow the highest possibilities for alterations in both the basic structure and its substituents. The complexity of total synthesis of this size of molecules which generally needs more than 15 steps, may be a major obstacle for evaluation of any further analogs that might be obtained in this way.

1.4 Total synthesis of daunomycin and analogs.

Although most of the analogs of daunomycin and adriamycin have been prepared by semi-synthetic methods, these methods will not be reviewed here, as they are beyond the scope of this thesis. When total synthesis of daunomycin analogs is indicated, it implies synthesis of both the aglycone and the sugar part, and then coupling of the protected sugar with the aglycone followed by deprotection.

Coupling of the protected sugar was originally achieved via the well known Koenings-Knorr method[11] in which a labile halo-daunosamine is coupled to the aglycone using a mixture of mercury salts ($HgO/HgBr_2$). A modification to this method was published by Arcamone *et al.*[12], in which silver triflate was used instead of mercury salts.
Both methods gave predominately the desired α-glycoside, but also some of the undesired β-glycoside. A third method was published in 1984 by Terashima and colleagues[13]. They used

trimethylsilyl triflate and a stable protected sugar with *p*-nitrobenzyl groups at the 1 and 4 positions. Only the desired α-glycoside was formed using this method and it is now the most commonly used method for the synthesis of daunomycin and its analogs.

Synthesis of the sugar daunosamine has been studied by many groups. A review article[14] shows many possibilities to synthesize daunosamine from cheap chiral (carbohydrate) precursors. We have applied the synthesis method from the commercially available α-methyl-D-manno-pyranoside as published by Horton and Weckerle[15], with some modifications.

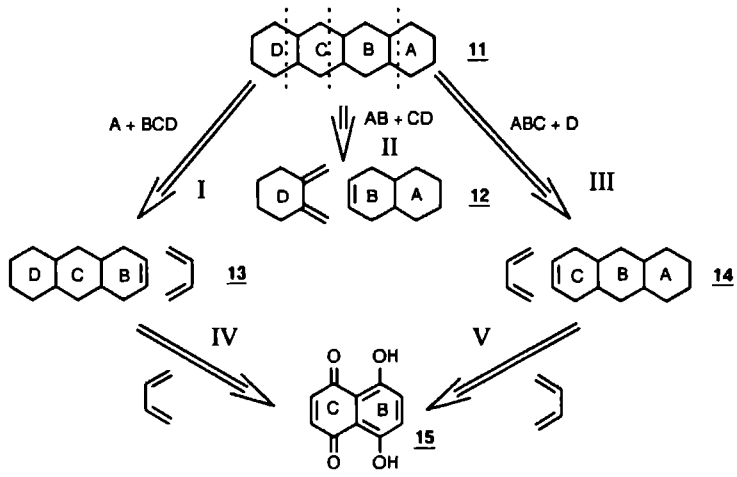
Synthesis of the aglycones of anthracyclines (anthracyclinones) are, in the first instance, directed to daunomycinone-type aglycones which can easily be transformed into adriamycinone type aglycones[1a] by bromination and subsequent substitution of the bromine atom by a hydroxy group.

Excellent reviews have been published on the synthesis of anthracyclinones[16a-d]. Krohn[16c] divided the described methods into four main type of reactions:

1 Friedel-Crafts;
2 Anionic;
3 Diels-Alder;
4 Transition-metal-mediated.

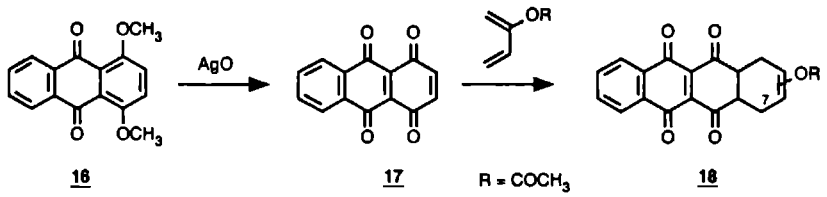
As our approaches to the construction of the anthracyclinones involve Diels-Alder reactions, the Diels-Alder strategies previously described by others are surveyed in this Chapter with special attention to those strategies that are important for our approach[17a-c]. A priori, several Diels-Alder routes are possible and the more important routes are shown in Figure 1.3 in a conceptual fashion.

Figure 1 3



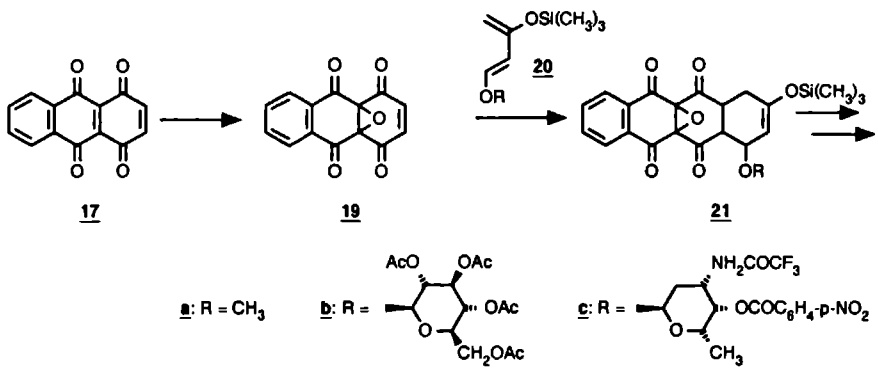
The first route (I, Figure 1.3, the DCB + A approach) was used by Kende *et al.*[18] and Lee *et al.*[19]. These two groups presented, independently at the same time, the first Diels-Alder approach leading to a useful synthesis of (4-demethoxy)daunomycinone. The Diels-Alder reaction of the quinizarinequinone **17** (obtained from dimethoxyanthraquinone **16**) with 2-alkoxybutadiene gave product **18** (mixture of regioisomers), which had to be functionalized at C-7 (Scheme 1.1). This functionalization is possible but rather cumbersome, and yields of not more than 50% were achieved[18a].

Scheme 1.1



To circumvent functionalization at C-7 at a later stage, Stoodley[20] used 1-methoxy-3-trimethylsilyloxybuta-1,3-diene (**20a**) in the Diels-Alder reaction with **19** (prepared from **17** by selective oxidation). To prevent cycloaddition to the internal double bond of **17**, this bond had to be protected. Product **21** was converted into 7-O-methyl-4-demethoxydaunomycinone. Removal of the methyl group from the methoxy function at the 7 position by solvolysis in trifluoroacetic acid was possible, but chromatographic separation of the mixture consisting of cis- and trans-7,9-dihydroxy isomers appeared to be extremely tedious[21].

Scheme 1.2



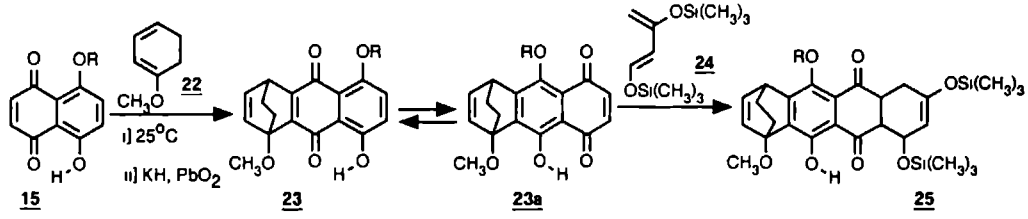
Two years later, Stoodley[22] was able to synthesize (+)-4-demethoxydaunomycinone using diene **20b**, made from optically active 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide.

Recently, Brasca *et al.*[23] described a method for synthesis of (+)-4-demethoxydaunomycinone using chiral diene **20c**. Protected daunosamine was used as chiral auxiliary and no glycosidation of the aglycone was necessary. The disadvantages of these methods are the restricted availability of the dienophile (substituted quinizarinequinones are very difficult to synthesize and to epoxidize) and the reduced reactivity of dienes **20b** and **20c** compared with diene **20a**. The dienes **20b** and **20c** react only with activated dienophiles, such as **19**. It appeared, in our hands, not sufficiently reactive with quinones, such as naphthazarin **15** (Scheme 1.3).

Potman[24] tried to synthesize several analogs of the quinizarinequinone which could be used for the synthesis of D ring analogs of daunomycinone, but either synthesis or oxidation of the dimethoxyanthraquinones analogs was not possible. Therefore, he concluded that this approach is a straightforward route for the synthesis of 4-demethoxydaunomycinone but the scope of the method is seriously limited by the difficult availability of suitable substituted quinizarinequinones.

A second method published by Kelly *et al.*[25] (A + BCD approach) includes steps IV and I (Figure 1.3) to synthesize daunomycinone in a regioselective manner starting from commercially available naphthazarin **15a** (Scheme 1.3). After protection of one of the hydroxy groups of **15b**, the daunomycinone skeleton **25** is constructed via two subsequent Diels-Alder reactions with 1-methoxycyclohexadiene **22** and 1,3-bistrimethylsilylbuta-1,3-diene **24**, respectively.

Scheme 1 3

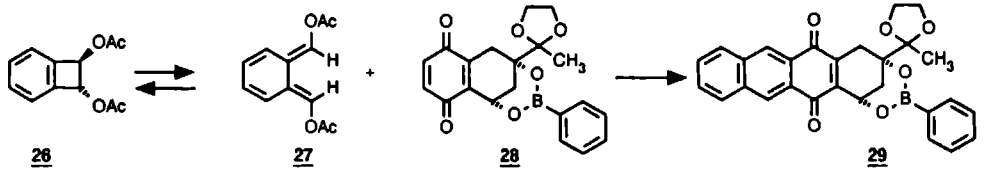


a R= H **b** R = $COOCH_2C_6H_4$ p NO_2

Synthesis of other D ring derivatives is possible but limited by the availability of the appropriate cyclohexadienes.

Several methods for the synthesis of daunomycinone via route II (Figure 1.3, the AB + CD approach) have been described. Broadhurst[26] reported a multigram synthesis of 4-demethoxy-daunomycinone by using dienophile **28** and diene **27**, which had been generated *in situ* (Scheme 1.4). The method is limited by the rather difficult synthesis of the dienophile **28**.

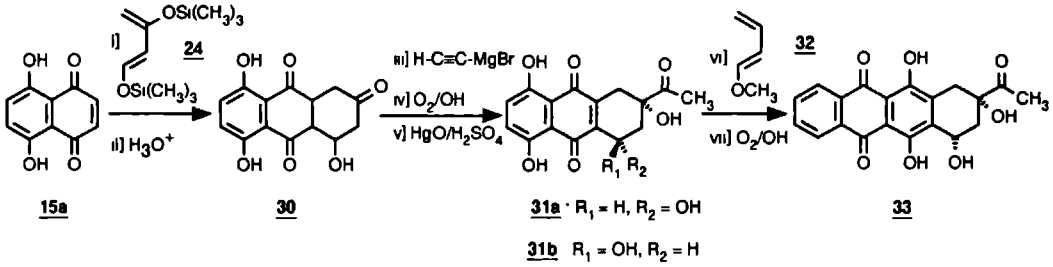
Scheme 1 4



26 27 28 29

An approach via route III and V (Figure 1.3) was developed by Krohn *et al.*[27] for the synthesis of 4-demethoxydaunomycinone. Naphthazarin **15a** was used as the BC fragment, which was converted into cycloadduct **30** with 1,3-bistrimethylsilyloxybuta-1,3-diene **24** (Scheme 1.5). After functionalization of ring A (**30** to **31a**) and a second Diels-Alder reaction with 1-methoxybutadiene **32**, 4-demethoxydaunomycinone **33** was formed in an overall yield of 29%. By using suitable dienes, this method opens a way for synthesizing a variety of D ring functionalized daunomycinone analogs. Limitations of this method are the conversion of **30** into **31a**, which is not stereospecific, and separation of the formed cis- and trans-hydroxy compounds **31a** and **31b** by preparative thin-layer chromatography. Analogous problems are encountered in further functionalization of **25** (Scheme 1.3). Furthermore, no regioselectivity is expected in conversion of **31** to daunomycinone derivatives with a functionalized D ring.

Scheme 1 5



15a 30 31a · R_1 = H, R_2 = OH 31b R_1 = OH, R_2 = H 33

The strategies for the synthesis of the aglycones described in this thesis are based on the methods of Kelly *et al.*[25] and Krohn *et al.*[27]. Limitations of these described methods have been overcome by several modifications and changes of essential steps, so that it is now possible to synthesize the anthracyclinones on a gram scale.

1.5 Outline of this thesis.

This thesis describes the total synthesis of known and some new daunomycin analogs and evaluation of the anti-tumor activity of the new analogs in order to find analogs with higher therapeutic indices. Regioselective syntheses of the anthracyclinone part have been developed which allow substituent variations in the A and D rings. An approach is also presented for introducing the necessary chirality into ring A via cycloaddition reactions.

In Chapter 2, multigram syntheses of daunomycinone and 4-demethoxydaunomycinone via the A + BCD approach are described which are based on the method of Kelly *et al.*[25] (steps IV and I, Figure 1.3). To make this method more practical, essential steps were modified and a step which is not reproducible has been circumvented. Chapter 3 deals with the synthesis of daunomycinone and 4-demethoxydaunomycinone via the ABC + D approach (steps V and III, Figure 1.3). This route was first applied by Krohn *et al.*[27] to synthesize anthracyclinones in a non-regioselective way. This method has been made more stereoselective by changing the used diene and the Grignard reagents. Regioselectivity is obtained by regioselective protection of the ABC fragment.

In Chapter 4, an approach is studied for obtaining optically active aglycones by using chiral 1-oxysubstituted-3-trimethylsilyloxybuta-1,3-dienes. The preparation of the dienes and their cycloadditions with naphthazarin, juglone, naphthoquinone and benzoquinone are described. Diastereomeric excesses up to 99% are accomplished depending on the dienes and quinones used. A model is presented to explain the diastereomeric excess observed.

Chapter 5 describes the synthesis of C_{13},C_{14}-ethynyl analogs of daunomycin and 4-demethoxy-daunomycin. The necessary racemic anthracyclinones are prepared according to methods described in Chapter 2. Glycosidation with the chiral protected sugar, daunosamine, followed by separation of the diastereomers and deprotection, yields the chiral ethynyl analogs.

The synthesis of pyridine D ring analogs of daunomycin are described in Chapter 6. The followed route towards the anthracyclinone is an extension of the method described in Chapter 3. The pyridine D ring is constructed via a Diels-Alder reaction of the ABC fragment with 1-dimethylamino-3-methyl-1-aza-1,3-butadiene. Coupling of the pyridine-containing anthra-cyclinone with the sugar moiety and isolation of the enantiopure D ring derivative is analogous to the preparation of the ethynyl analogs as described in Chapter 5.

Chapter 7 gives an overview of the current status with respect to anti-tumor activity and mechanisms of action of daunomycin and its known derivatives. Five mechanisms of action are described which are proposed to contribute to the cytotoxicity effect. Factors that may cause cardiotoxicity are also presented. An overview is then given of derivatives that are currently in clinical use or under evaluation. The results of systematic modification at four different sites of the anthracyclines, e.g., C_{13},C_{14}-side chain (A ring), BC rings, D ring and modifications in the sugar part are discussed.

In Chapter 8, the results of biological tests of the newly synthesized daunomycin analogs are evaluated. The ethynyl analogs (Chapter 5) and the pyridine analogs (Chapter 6) have been tested against five human tumor cell lines for in vitro cytotoxicity. The test results of the four most active compounds (in vitro) with respect to their acute toxicity (MTD and lethal dose) are also described. For the same four compounds, the in vivo L-1210 tests are also presented. Finally for these derivatives, the results of an in vitro cardiotoxicity test are given. The Chapter ends with a critical evaluation of the obtained test results. The thesis is concluded with summaries in English and Dutch.

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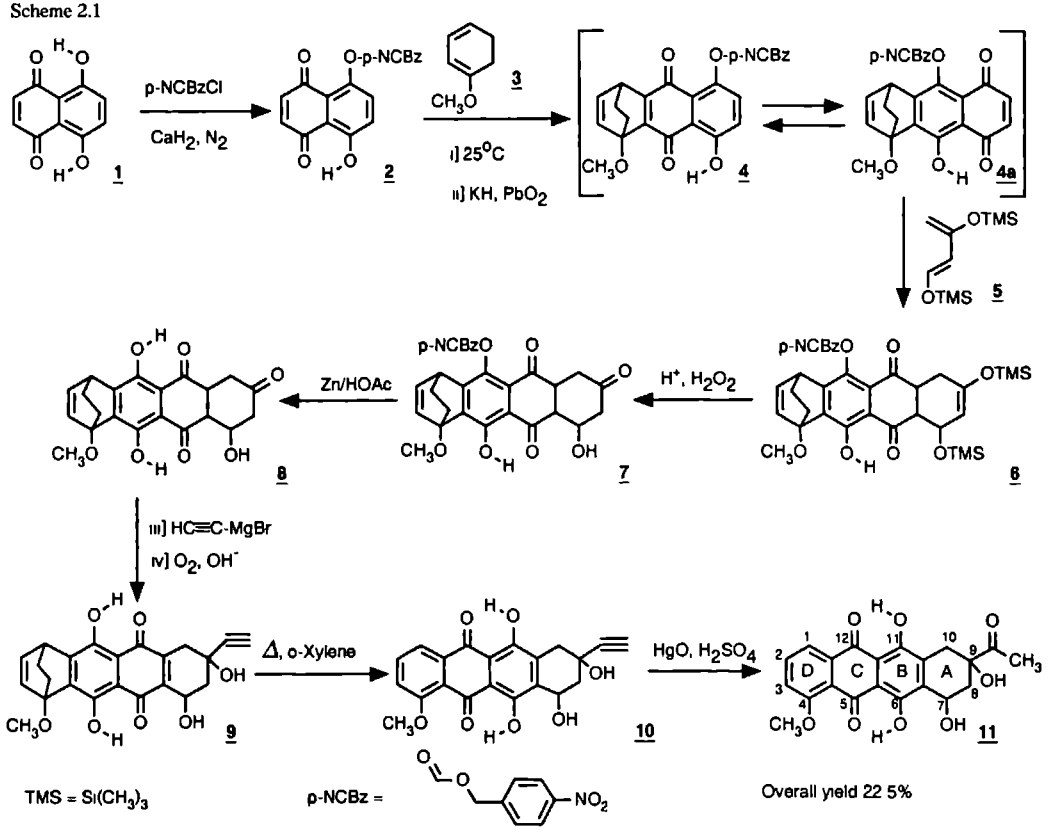
CHAPTER 2

A + BCD APPROACH TO DAUNOMYCINONE AND DERIVATIVES.

2.1 Introduction.

This chapter describes the synthesis of both symmetrical and non-symmetrical D ring derivatives of daunomycinone via the A + BCD approach, as outlined in the preceding chapter. Kelly *et al.*[1] used this approach for the synthesis of (+/-)-daunomycinone 11 (Scheme 2.1). Some fundamental improvements to this approach will be described, which allow synthesis of (+/-)-daunomycinone and its analogs on a multigram scale.

Scheme 2.1

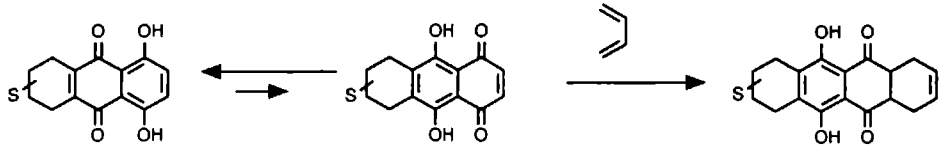


Kelly's first approach to the daunomycinone type of compounds was suggested by two earlier published examples.

Fariña *et al.*[2] indicated that substituted naphthazarins exist primarily in the tautomeric form 12,

which is favored, but there is an equilibrium with tautomeric form **12a** which can be trapped in a Diels-Alder reaction (Scheme 2.2).

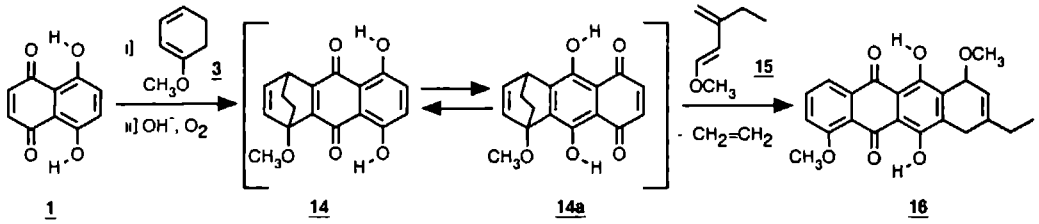
Scheme 2.2



12 **12a** **13**

Together with a method for the synthesis of polycyclic quinones via Diels-Alder addition of 1-methoxycyclohexa-1,3-diene to a quinone (Scheme 2.3, **1** and **3**), followed by oxidation, this Diels-Alder reaction of the tautomerized product (Scheme 2.3, **14a** + **15**) and thermal elimination of the ethano bridge, afforded the concept for the synthesis of daunomycinone **11**[2] from napthazarin[3]. In this way, the daunomycinone skeleton can be built up in four steps (Scheme 2.3).

Scheme 2.3



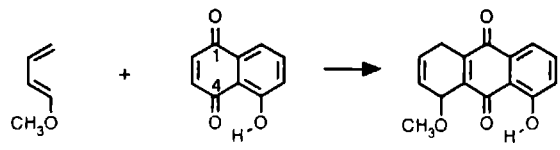
1 **14** **14a** **16**

Unfortunately, mainly the undesired regioisomer of the biologically active daunomycinone is formed and, therefore, Kelly and colleagues developed a method in which the regioselectivity was reversed.

To achieve this reversal, two important concepts were applied:

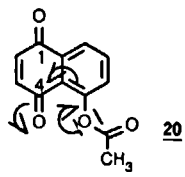
- the reaction of juglone **18** with 1-methoxybutadiene **17** afforded a regioselective adduct **19** (Scheme 2.4) because of the H-bonding interaction, which makes the C-4 carbonyl group more electron-poor than the C-1 carbonyl[3].
- acetylation of the hydroxy group (Figure 2.1, **20**) reverses the selectivity, although not completely[4]. The electron-donating properties of the acyloxy-oxygen make the C-4 carbonyl more electron-rich.

Scheme 2.4

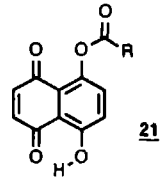


17 **18** **19**

Figure 2.1



20



21

The combination of these effects, H-bonding interaction of the free hydroxy group and acetylation of the other hydroxy group by an RCO group (as indicated in Figure 2.1, 21) gave complete regioselectivity in the cycloaddition of 2 (with 3) and 4a (with 5, Scheme 2.1).

- the second important concept for the synthesis of daunomycinone was "acyl-wanderung". In order to use the protecting group for both cycloadditions with full regioselectivity, the RCO group must be transferred intramoleculary (Scheme 2.1, equilibrium between 4 and 4a). This principle of "acyl-wanderung" had been observed earlier by Brockmann *et al.*[5] and Alvarado *et al.*[6]. Kelly used it for the equilibrium between 4 and 4a.

A key step in the Diels-Alder approach is the use of 1-alkoxy-3-trimethylsilyloxy-1,3-butadienes or 1,3-bis(trimethylsilyloxy)-1,3-butadiene (Scheme 2.1, 5). These dienes offer the possibility for functionalizing the A ring of the molecule at an early stage. The ketone group of 8 can easily be transferred into the necessary α-hydroxy,acetyl functionality at the 9 position of daunomycinone 11 (Scheme 2.1). The free hydroxy group at the 7 position is obtained by hydrolysis of the alkoxy group of the diene.

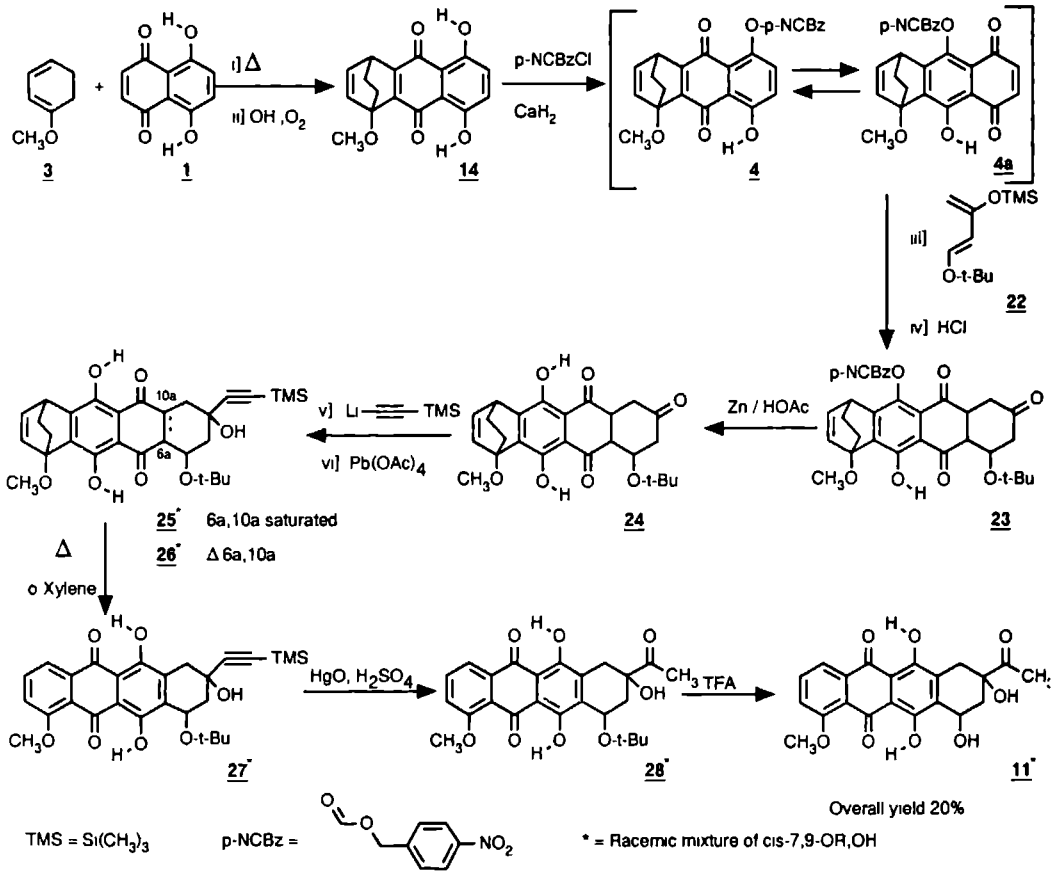
In trying to repeat the sequence as described by Kelly, we encountered three serious problems:

- we were not able to reproduce oxidation of the cycloadduct of dienophile 2 and diene 3 (Scheme 2.1). Kelly and his group[7] also reported problems with the use of KH/PbO_2 and in isolating 4 (Scheme 2.1) in acceptable yields. The particle size of commercial KH seemed to be very important. The amount of KH was adjusted to 0.05 equivalent, which made the reaction much slower but suppressed cleavage of the protective group, which had become a major side reaction. We were not able to repeat this procedure and we always found cleavage of the protecting group. In addition, we tried a variety of other oxidation methods without success.
- another problem in this synthesis of daunomycin is the reaction with the Grignard reagent (steps 8 to 9, Scheme 2.1) in order to convert the ketone into a α-hydroxy, α-ethynyl functionality. Firstly, the necessary use of 30 equivalents of ethynyl magnesium bromide is very inefficient. Furthermore, we encountered serious problems with upscaling the reaction in this and similar examples. The yields were reasonable at the 100 mg scale but dropped dramatically when performing the reaction at a gram scale[8].
- finally, the formation of both cis- and trans-7,9-dihydroxy compounds 9 is unsatisfactory as it necessitates subsequent difficult separation of diastereomers.

2.2 Synthesis of (+/-)-daunomycinone.

We have developed a gram scale synthesis of (+/-)-daunomycinone via the Diels-Alder approach taking advantage of Kelly's method but circumventing the disadvantages, as shown in Scheme 2.5.

Scheme 2 5

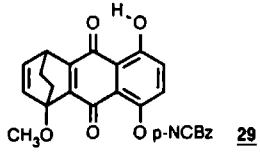


First, we tried other protecting groups for 1 (Scheme 2.5) which would give the desired "acyl-wanderung". Only Kelly's 4-nitrobenzyloxycarbonyl group appeared to be successful. Other ester or carbamate groups could be attached to naphthazarin 1 but neither oxidation nor intramolecular shift was achieved.

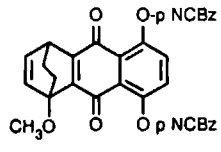
Finally we decided to change the order of the reaction steps and to protect the oxidized cycloadduct 14 (Scheme 2.5) instead of oxidizing the protected cycloadduct. Of the protective groups we tried (i.e., acetate, benzoate, benzyloxycarbonyl and mandelate), only the *p*-nitro-carbobenzoxy group was successful. Three possible products can be formed (14, 29 and 30). In order to find optimal selectivity for this reaction, we tried several bases, such as NaH, KO-*t*-Bu, Na_2CO_3, DABCO and CaH_2 and we made numerous attempts in varying the equivalents of base

and *p*-nitrobenzyl chloroformate. This reaction appeared to be quite successful when 1 equivalent of the protecting agent and 1.6 equivalents of calcium hydride were used. Only minor amounts of the undesired mono-protected **29** (Figure 2.2) and di-protected cycloadducts **30** (Figure 2.2) were formed. Some unreacted naphthazarin was also recovered after column chromatography.

Figure 2 2



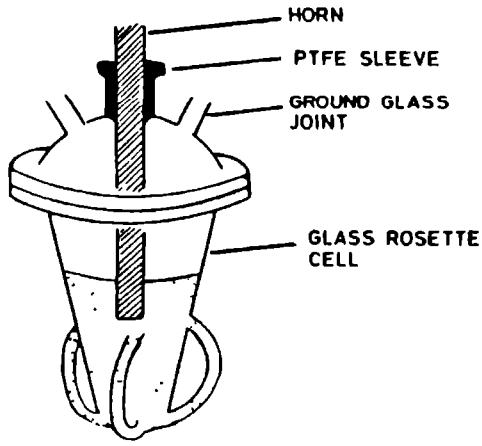
29



30

Initially, the reaction was very slow (6 days at room temperature, conversion 30-40%), but acceleration was accomplished by the using a Direct Immersion Sonic Horn[9] (Cell disrupter) and a glass rosette cell (Figure 2.3) and/or an ultrasonic cleaning bath. The reaction time decreased to 30 h and the conversion increased to 75-80%. The isolated yield after column chromatography was 50%. All the side products formed are easily reconverted into naphthazarin and can be used again.

Figure 2 3



The mono-protected ethanoanthracene derivative **4**/**4a** underwent a Diels-Alder reaction with 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene **22** to give the adduct **23** in a yield of 80% (Scheme 2.5).

Diene **22** was synthesized according to the method of Potman *et al.*[10] or according to the method of Danishefsky *et al.*[11] (Scheme 2.6).

The advantages of using the *tert*-butoxy diene **22** instead of the trimethylsilyloxy group (**5**, Scheme 2.1) are stability of the cycloadduct and high stereoselectivity for the subsequent introduction of the acetylene functionality. We found that, when the trimethylsilyloxy group is

deprotected in an early stage (conversion 6 to 7, Scheme 2.1), partial aromatization to compound 38 cannot be avoided and purification of the reaction mixtures is almost impossible (as is also the case in Kelly's method).

Scheme 2 6

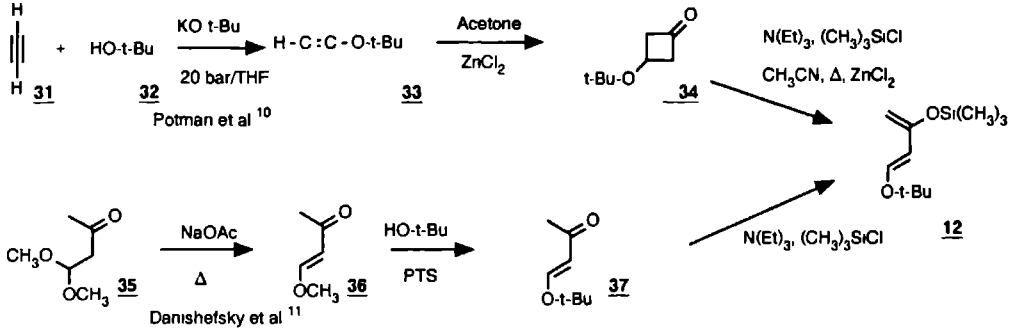


Figure 2 4

Introduction of the ethynyl functionality in compound 24 via the Grignard reaction in the desired manner trans to the *tert*-butoxy group occurs with a higher stereoselectivity than in compound 8 trans to the hydroxy group[12]. However, partial aromatization to compound 38 cannot be avoided and purification of the reaction mixture is almost impossible (as is also the case in Kelly's method).

A major breakthrough was the use of 2-trimethylsilylethynyllithium[13]. No more than 6 equivalents were necessary and formation of 38 (Figure 2.4) is strongly suppressed. Clearly, the trimethylsilyl acetylide behaves more as a nucleophile and less as a base. We also found that no better results were obtained when using cerium acetylides as described for related reactions[13]. When using 2-trimethylsilylethynyllithium, only the cis-7-*t*-butoxy-9-hydroxy-isomer could be detected by ^{1}H-NMR spectroscopy. The fact that only the cis isomer is formed is due to the quasi-axial position[8] of the *tert*-butoxy and the bulkiness of the 2-trimethylsilylethynyl group. Separation of the (+/-)-daunomycinone and its (+/-)-7-epimer is then no longer necessary. The reaction was performed at a 5-gram scale and the yields were comparable with the reaction at the 100 mg scale. Further upscaling should, therefore, be possible.

The isolation of pure product 25 was very difficult because of the formation of some bis adduct, probably 39 (Figure 2.4), and spontaneous air oxidation to product 26. After oxidation with $Pb(OAc)_4$ and retro-Diels-Alder reaction in *o*-xylene, we were able to obtain compounds 27 by crystallization from diethyl ether (yield 74%).

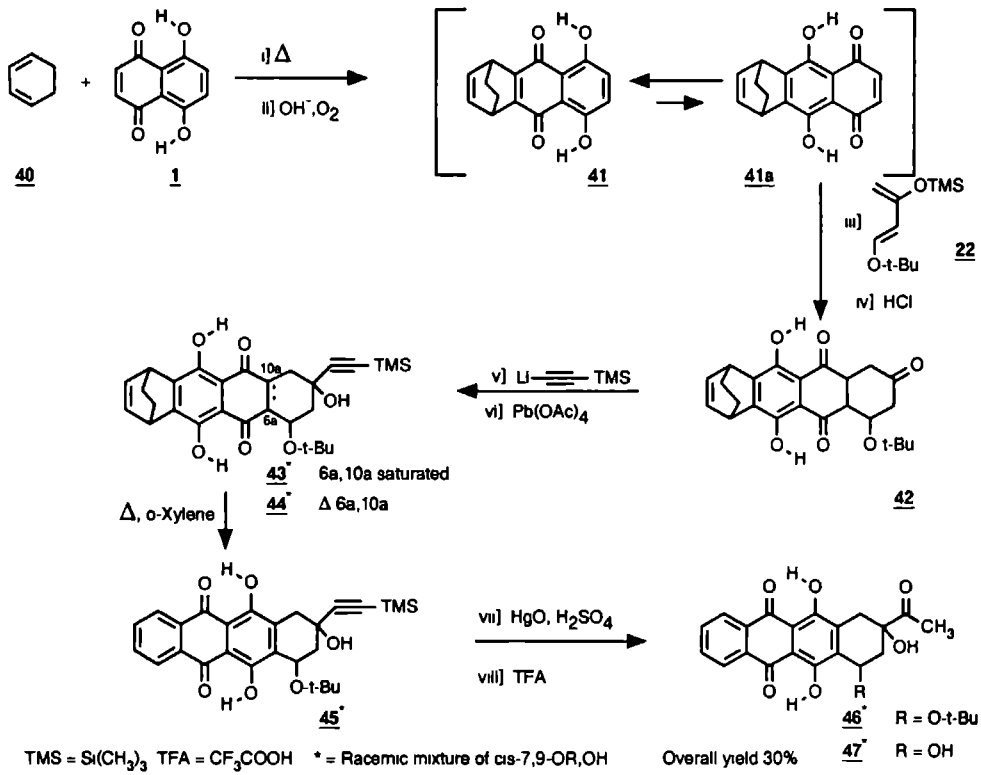
Transformation of the 2-trimetylsilylethynyl functionality (Scheme 2.5, 27 to 28) to the acetyl group has been done by simple treatment with mercury (II) oxide in dilute sulfuric acid in tetrahydrofuran. Because of the higher reactivity of the 2-trimethylsilylethynyl functionality and better solubility of compound 27, compared to the unsubstituted ethynyl compound 10, the reaction is much faster (3 h and 1 night, respectively) and gives better yields (91% and 75%, respectively).

After treatment with trifluoroacetic acid at room temperature, product 28 gives (+/-)-daunomycinone 11 in an overall yield of 21%. The obtained (+/-)-daunomycinone after recrystallization from chloroform, exhibits spectral and physical properties identical to those reported previously (Scheme 2.5).

2.3 Synthesis of (+/-)-4-demethoxydaunomycinone.

The advantages of using 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene 22 and lithium trimetylsilylacetylide are generally applicable to the synthesis of anthracyclinones of the daunomycinone type. This is further illustrated in the synthesis of 4-demethoxydaunomycinone (Scheme 2.7).

Scheme 2.7



The Diels-Alder reaction of naphthazarin **1** with cyclohexa-1,3-diene **40** was, as expected, much slower than in the former case with 1-methoxycyclohexa-1,3-diene **3**. Compounds **40** and **1** had to be refluxed for 5 days in tetrahydrofuran to yield 75% of **41**.
As regioselectivity does not have to be controlled, the second Diels-Alder reaction of **41a** with the 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene **22** leads to the daunomycinone skeleton in two steps (Scheme 2.7).
The ABCD fragment was functionalized by the use of 2-trimethylsilylethynyllithium to give product **43** with the desired high cis stereoselectivity of the C_9 OH group and the C_7 O-*t*-Bu group. After reaction with 2-trimethylsilylethynyllithium the crude product (no purification) was oxidized with $Pb(OAc)_4$ and subsequently purified by column chromatography to give **44** (76%). A retro-Diels-Alder reaction in refluxing *o*-xylene afforded compound **45** in 91%. This compound could be easily converted into (+/-)-4-demethoxydaunomycinone **47** by hydration with mercury(II) oxide in dilute sulfuric acid/tetrahydrofuran and subsequent treatment with trifluoroacetic acid. The overall yield was 30%.

2.4 Conclusions.

A multigram synthesis for (+/-)-daunomycinone and (+/-)-4-demethoxydaunomycinone has been described. To accomplish this, in essence the method of Kelly *et al.* was used starting from naphthazarin by the A + BCD approach (Chapter 1, Figure 1.3) but, essential steps were modified. In order to circumvent the tedious oxidation step of using potassium hydride and lead(IV) oxide, first, the Diels-Alder adduct was formed. This adduct was easily oxidized by oxygen under basic conditions and could then be protected. The Grignard reaction with ethynyl magnesium bromide was also adapted. By using 2-trimethylsilylethynyllithium, the reaction was more efficient (only 6 equivalents of reagent was needed in stead of 30 equivalents of ethynyl magnesium bromide). Now it was possible to accomplish this reaction at a gram scale. Application of 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene, instead of the 1,3-bis-trimethylsilyloxybuta-1,3-diene, together with the use of 2-trimethylsilylethynyllithium resulted in the exclusive formation of the desired cis-7-*t*-butoxy-9-hydroxy-isomer.
(+/-)-Daunomycinone and (+/-)-4-demethoxydaunomycinone were isolated on a gram scale with an overall yield of 20% and 30%, respectively. In principle this method will also allow the synthesis of other symmetric or non-symmetric, D ring derivatives of daunomycinone. The scope of these reactions depends on the availability and the reactivity of the cyclohexadienes which are used.

2.5 Experimental section.

General remarks:
^{1}H-NMR spectra were measured on a Bruker WH-90 spectrometer using $(CH_3)_4Si$ as internal standard. $CDCl_3$ was used as solvent. Melting points were taken using a Reichert Thermpan

microscope and are uncorrected. IR spectra were obtained using a Perkin Elmer 298 infrared spectrometer. Mass spectra were taken using a double focussing VG 7070 E mass spectrometer. For column chromatography, either Merck Silicagel 60 or Merck Silicagel Art. 9385 (flash chromatography) were used.

1,4,9,10-Tetrahydro-5,8-dihydroxy-1-methoxy-9,10-dioxo-1,4-ethanoanthracene (14).

To a solution of 20.9 g (0.11 mol) of 5,8-dihydroxy-1,4-naphthoquinone 1 in 330 ml of dichloromethane were added 28.4 g of 1-methoxycyclohexa-1,3-diene 3 (technical grade 65%, 0.177 mol). The mixture was refluxed for 6 h. In order to circumvent formation of by-products, the temperature of the oil-bath did not exceed 50°C. The solvent was evaporated and the residue stirred with 330 ml of pentane for 5 minutes. The solution was then cooled to 0°C and the yellow/brown solid (30.0 g, 91%) was collected by filtration, rinsed with petroleum ether (40/65) and directly used in the next reaction.

The crude product was dissolved in a solution of 22.0 g (0.55 mol) of sodium hydroxide in 825 ml of water. Air was passed through the solution and the color of the mixture changed from green to blue. After completion of the reaction, monitored by TLC (Eluent ethyl acetate/n-hexane, 2 : 5), 46.2 ml of concentrated HCl (37%) were added. The product precipitated as a red solid and it was filtered off and then dried *in vacuo* over P_2O_5 to give 27.0 g of 14 (83% overall from 1). A sample of product 14 was re-crystallized in ethyl acetate/n-hexane to give yellow needles of 14; m.p. 169-171°C (dec., Lit.[14] 170°C, Lit.[15] 167-169°C). MS., m/e (EI) 298, 283, 270, 252, 240, 224; ^{1}H-NMR ($CDCl_3$, 90 MHz): δ = 1.39-1.92 ppm (4H, m, CH_2-CH_2), δ = 3.69 ppm (3H, s, OCH_3), δ = 4.47-4.61 ppm (1H, m, H_4), δ = 6.40 ppm (1H, dd, J=5.6 Hz and J=8 Hz), δ = 6.64 ppm (1H, dd, J=8 Hz and J=1.5 Hz), δ = 7.12 ppm (2H, s, ArH), δ = 12.60 ppm (1H, s, ArOH), δ = 13.06 ppm (1H, s, ArOH). Anal. calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.01; H, 4.77.

1,4,9,10-tetrahydro-8-hydroxy-1-methoxy-5-(4-nitrobenzyloxycarbonyloxy)-1-methoxy-9,10-dioxo-1,4-ethanoanthracene (4).

To 12.0 g (0.04 mol) of 14, dissolved in 300 ml of dry THF (distilled from sodium benzophenone ketyl) were added 12.6 g (0.062 mol) of *p*-nitrobenzyl chloroformate and 1.7 g (0.04 mol) of powdered calcium hydride. The suspension was sonicated with a Direct Immersion Sonic Horn[9] (Cell disrupter) in a glass rosette cell[9] at 0°C for 6 hours in order to activate the calcium hydride. The mixture was then poured into a 500 ml round bottom-flask and sonicated for 24 h in an ultrasonic cleaning bath. The color of the solution changed from red to yellow and progress was monitored by TLC (ethyl acetate/n-hexane, 2 : 5). After the reaction was almost complete, the reaction mixture was quenched by addition of 500 ml of an aqueous solution of 5% NaH_2PO_4 and the mixture was then extracted twice with 500 ml of chloroform. The chloroform extract was washed with brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The crude product was purified by flash column chromatography (silicagel, ethyl acetate/ toluene/n-hexane, 1 : 5 : 5). After concentration *in vacuo*, the solid residue was stirred overnight in 200 ml of diethyl ether. Filtration gave 9.6 g (50%) of pure 4. m.p. 136-137°C (Lit.[1] 115-116°C, recrystallized from ethyl acetate/diethyl ether); MS, m/e (Cl^+) 478, 450, 405, 299, 271;^{1}H-NMR (90 MHz, $CDCl_3$) : δ = 1.33-1.89 ppm (4H, m, CH_2-CH_2), δ = 3.66 ppm (3H, s, OCH_3), δ = 4.30-4.47 ppm (1H, m, H_4), δ = 5.44 ppm (2H, s, CH_2), δ = 6.34 ppm (1H, dd, J=5.6 Hz and J=8 Hz, H_3), δ = 6.61 ppm (1H, dd, J=8 Hz and J=1.5 Hz, H_2), δ = 7.28 ppm (2H, s, ArH), δ = 7.54 ppm (2H, AB, J=8.5 Hz, ArH), δ = 8.26 ppm (2H, AB, J=8.5 Hz, ArH), δ = 12.72 ppm (1H, s, ArOH). Anal. calcd. for $C_{25}H_{19}NO_9$: C, 62.89; H, 4.01; N, 2.93. Found: C, 62.41; H, 3.92; N, 2.95. IR (KBr): 1765 cm^{-1}.

1-tert-Butoxy-3-trimethylsilyloxybuta-1,3-diene (22).

Compound 22 was prepared as described by Potman *et al.*[10] from 101 g of 1-*tert*-butoxy-1-buten-3-one. Yield 107.5 g (71%), b.p. 57-60°C/0.75 Torr (Lit[10] 50-53°C/0.25 Torr).

7-tert-Butoxy-1,4,6a,7,8,10a-hexahydro-5-hydroxy-4-methoxy-12-(4-nitrobenzyloxycarbonyloxy)-6,9,11-(10H)-trioxo-1,4-ethanonaphthacene (23).

To a solution of 7.5 g (0.016 mol) of **4** in 175 ml of dry (distilled from sodium benzophenone ketyl) THF under an argon atmosphere, 6.75 g (0.032 mol) of 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene **22** were added and the reaction mixture was stirred at room temperature for 2 days. The color of the solution changed from yellow/orange to yellow/green. The reaction was monitored using TLC (ethyl acetate/n-hexane, 2 : 5). Subsequently, the cycloadduct was hydrolysed by cooling the reaction mixture to 0°C and adding 7.5 ml of 1N HCl. After 15 minutes, 175 ml of water were added and the mixture was extracted twice with 125 ml of dichloromethane. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded the crude product, which was then stirred overnight in 300 ml of diethyl ether. Filtration gave 7.8 g (80%) of product **23**. m.p. 147-150°C; MS, m/e (CI) no mol mass peak found, 440; ^{1}H-NMR (90 MHz, $CDCl_3$, mixture of isomers, ratio 7 : 3): δ = 0.74 ppm (9H, s, $C(CH_3)_3$), δ = 1.43-1.93 ppm (4H, m, CH_2-CH_2), δ = 2.08-2.62 ppm (3H, m, H_{10}(ax), H_8(ax) and H_8(eq)), δ = 3.23-3.70 ppm (3H, m, H_{6a}, H_{10}(eq) and H_{10a}), δ = 3.72 and 3.75 ppm (3H, 2 x s, OCH_3), δ = 4.11-4.26 ppm (1H, m, H_7), δ = 4.43-4.56 ppm (1H, brs, H_1), δ = 5.42 ppm (2H, brs, CH_2), δ = 6.26-6.80 ppm (2H, m, H-C=C-H), δ = 7.56-8.33 ppm (4H, 2 x AB, ArH), δ = 13.19 and 13.23 ppm (1H, 2 x s, ArOH). Anal. calcd. for $C_{33}H_{33}NO_{11}$: C, 63.97; H, 5.37; N, 2.26. Found: C, 63.33; H, 5.27; N, 2.26. IR (KBr): 1770 (carbonyl carbonate), 1720 (A ring carbonyl) and 1630 (quinone carbonyl).

7-tert-Butoxy-1,4,6a,7,8,10a-hexahydro-5,12-dihydroxy-4-methoxy-1,4-ethanonaphthacene-6,9,11-(10H)-trione (24).

To a stirring solution of 5.0 g (0.008 mol) of **23** in 20 ml of THF, 20 ml of glacial acetic acid and 5.2 g (0.08 mol) of zinc were added. The zinc was first activated by the procedure described by Perrin *et al.*[16]. After 15 min another 5.2 g of zinc were added. Progress of the reaction was monitored by TLC (ethyl acetate/n-hexane, 3 : 5) until the reaction was complete (approx. 30 min). The mixture was then diluted with 250 ml of dichloromethane and the acetic acid was neutralized (to pH 6) with saturated $NaHCO_3$. The layers were separated and the aqueous layer was extracted twice with 150 ml of dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was stirred overnight in 500 ml of dry diethyl ether. After cooling the solution to 0°C, solid **24** was filtered off (3.45 g, 97%). Analytically pure material was obtained as an off-white solid, m.p. 134-136°C by recrystallization from diisopropyl ether; MS, m/e (EI) 440, 412, 356, 336, 320; ^{1}H-NMR (90 MHz, $CDCl_3$, mixture of isomers, ratio 7 : 3): δ = 0.71 ppm (9H, s, $C(CH_3)_3$), δ = 1.38-1.89 ppm (4H, m, CH_2-CH_2), δ = 2.22-2.64 ppm (3H, m, H_{10}(ax), H_8(ax) and H_8(eq)), δ = 3.33-3.75 ppm (3H, m, H_{6a}, H_{10}(eq) and H_{10a}), δ = 3.71 and 3.75 ppm (3H, 2 x s, OCH_3), δ = 4.34-4.62 ppm (2H, m, H_1 and H_7), δ = 6.44 ppm (1H, dd, J=8 Hz and J=6.5 Hz, H-C=C), δ = 6.70 ppm (1H, dd, J=8 Hz and J=1.5 Hz, H-C=C), δ = 11.87 and 11.93 ppm (1H, 2 x s, ArOH), δ = 12.94 and 13.00 ppm (1H, 2 x s, ArOH). Anal. calcd. for $C_{25}H_{28}O_7$: C, 68.17; H, 6.41. Found: C, 67.91; H, 6.21. IR (KBr): 1720 (A ring carbonyl) and 1650 and 1620 (quinone carbonyl).

Cis-(+/-)-7-tert-butoxy-7,8,9,10-tetrahydro-4-methoxy-6,9,11-trihydroxy-9-[trimethylsilylethynyl]-5,12-naphthacenedione (27).

5.7 g (0.058 mol) of 2-trimethylsilylacetylene (96%) were dissolved in 750 ml of dry THF (distilled from sodium benzophenone ketyl) under an argon atmosphere. The reaction mixture was then cooled to -78°C and 37.9 ml of BuLi in n-hexane (1.5 M) were added dropwise over 10 min. The reaction mixture was stirred for 30 min at -78°C and then 5.0 g (0.0114 mol) of **24** were added. Progress of the reaction was monitored by TLC (ethyl acetate/n-hexane 2: 5) and the color of the solution changed from green to yellow/green). After 2 h, the reaction mixture was allowed to warm up to room temperature and 250 ml of a 10% aqueous solution of NH_4Cl were then added. The mixture was stirred for 15 min. 500 ml of water was added, and aqueous layer was extracted twice with 500 ml of chloroform. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate and then concentrated *in vacuo*.

The residue was dissolved in 100 ml of glacial acetic acid and 5.5 g (0.012 mol) of lead tetraacetate were added. The mixture was stirred overnight at room temperature. The color of

the mixture changed from yellow/red to red. The reaction mixture was then poured into 400 ml of water, the red solid which precipitated was filtered off and then dissolved in chloroform. The organic layer was washed twice with a solution of saturated sodiumhydrogen carbonate, washed with water and brine, dried over NaS_2O_4 and evaporated *in vacuo*. After column filtration (silicagel 60, 1:4, ethyl acetate/n-hexane) and evaporation of the solvent *in vacuo*, the solid residue was dissolved in 50 ml of *o*-xylene and refluxed for 3 h (temperature of the oil bath 150°C). After evaporation of the solvent, the product was recrystallized from dry diethyl ether to give 4.2 g (74%) of orange crystals **27**. m.p. 247-250°C; MS, m/e (EI) 508, 493, 452, 434, 416; ^{1}H-NMR (90 MHz, $CDCl_3$) : δ = 0,23 ppm (9H, s, $Si(CH_3)_3$), δ = 1.39 ppm (9H, s, $C(CH_3)_3$), δ = 1.99 ppm (1H, dd, J=15 Hz and J=3 Hz, H_8(ax)), δ = 2.72 ppm (1H, d, J=15 Hz, H_8(eq)), δ = 2.98 ppm (1H, d, J=20 Hz, H_{10}(ax)), δ = 3.64 ppm (1H, d, J=20 Hz, H_{10}(eq)), δ = 4.05 ppm (3H, s, OCH_3), δ = 5.30-5.40 ppm (1H, m, H_7), δ = 5.92 ppm (1H, s, 8-OH), δ = 7.35 ppm (1H, dd, J=8.25 Hz and J=1.0 Hz, H_3), δ = 7.74 ppm (1H, t, J =8 Hz and J=8.25 Hz, H_1), δ = 8.00 ppm (1H, dd, J=8 Hz and J=1 Hz, H_2), δ = 13.30 ppm (1H, s, ArOH), δ = 14.10 ppm (1H, s, ArOH). The compound was not obtained completely pure due to some elimination of the trimethylsilyl group.

*Cis-(+/-)-7-tert-butoxy-7,8,9,10-tetrahydro-9-acetyl-4-methoxy-6,9,11-trihydroxy-5,12-naphthacenedione (**28**).*

2.5 g (0.0049 mol) of **27** were dissolved in 120 ml of THF and 1.07 g (0.0049 mol) of HgO and 60 ml of 3M H_2SO_4 were added. The mixture was stirred for 3 h at room temperature, poured into 250 ml of 1N HCl and extracted three times with 250 ml of chloroform. After drying over anhydrous sodium sulfate and evaporation *in vacuo*, the solid was recrystallized from chloroform/ n-hexane to give 2.05 g (91%) of **28**. m.p. 244-245°C (dec.); ^{1}H-NMR (90 MHz, $CDCl_3$): δ = 1.39 ppm (9H, s, $C(CH_3)_3$), δ = 1.91 ppm (1H, dd, J=14.5 Hz and J=3 Hz, H_8(ax)), δ = 2.37 ppm (1H, brd, J=14.5 Hz, H_8(eq)), δ = 2.42 ppm (3H, s, CH_3), δ = 2.99 ppm (1H, d, J=19.2 Hz, H_{10}(ax)), δ = 3.26 ppm (1H, d, J=19.2 Hz, H_{10}(eq)), δ = 4.08 (3H, s, OCH_3), δ = 5.40-5.49 ppm (1H, m, H_7), δ =5.99 ppm (1H, s, 8-OH), δ = 7.36 ppm (1H, dd, J=7.5 Hz and J=1.0 Hz, H_3), δ = 7.75 ppm (1H, t, J =7.7 Hz and J=7.5 Hz, H_1), δ = 8.00 ppm (1H, dd, J=7.7 Hz and J=1 Hz, H_2), δ = 13.32 ppm (1H, s, ArOH), δ = 14.09 ppm (1H, s, ArOH).

*(+/-)-daunomycinone (**11**).*

2.05 g (0.0045 mol) of **28** were dissolved in 40 ml of trifluoroacetic acid and stirred for 10 min at room temperature. The reaction mixture was diluted with 100 ml of dichloromethane and extracted twice with 75 ml of water, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give 1.75 g (95%) of (+/-)-daunomycinone **11**. m.p. 212-214°C (lit 212-213°C); Spectral data were in accordance with earlier published[1].

*1,4,9,10-Tetrahydro-5,8-dihydroxy-9,10-dioxo-1,4-ethanoanthracene (**41**).*

19.0 g (0.1 mol) of 5,8-dihydroxy-1,4-naphthoquinone **1** and 12.5 g (0.16 mol) of cyclohexa-1,3-diene **40** were refluxed for 5 days in 190 ml of THF. After completion of the reaction, as was monitored by TLC (eluent; ethyl acetate/n-hexane, 2 : 5) and the color of the reaction mixture changed from red to yellow/brown, the solvent was removed *in vacuo* the residue was stirred in 200 ml of petroleum ether (40/65) for 1 h The solid was collected by filtration, rinsed with petroleum ether and used directly in the next reaction.

The yellow solid was added to a solution of 20.0 g of sodium hydroxide in 750 ml of water. The reaction mixture was stirred while air was bubbled through the solution. The reaction was monitored by TLC (ethyl acetate/n-hexane, 2 : 5). After 1 h, 35 ml of conc. HCl (37%) were added, the red solid which precipitated was filtered off and dissolved in chloroform. The organic layer was washed with saturated $NaHCO_3$, water, brine, dried over anhydrous sodium sulfate and then evaporated to give 20.1 g (75%) of a red solid residue **41**. m.p 203-204°C; MS, m/e (EI) 268, 240, 212, 183; ^{1}H-NMR (90 MHz, $CDCl_3$): δ = 1.16-1.58 ppm (4H, m, CH_2-CH_2), δ = 4.47-4.65 ppm (2H, m, H_1 and H_4), δ = 6.35 ppm (2H, dd, J=7.5 Hz and J=2.5 Hz, H-C=C-H), δ = 7.03 ppm (2H, s, ArH), δ = 12.48 ppm (2H, s, ArOH). Anal. calcd. for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.15; H, 4.47.

7-tert-Butoxy-1,4,6a,7,8,10a-hexahydro-5,12-dihydroxy-1,4-ethanonaphthacene-6,9,11-(10H)-trione (42).

To a solution of 19.4 g (0.072 mol) of **41/41a** in 300 ml of dry toluene were added 23.2 g (0.108 mol) of 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene **22** at room temperature and the reaction was stirred under an argon atmosphere for 5 days. After completion of the reaction, monitored by TLC (eluent; ethyl acetate/n-hexane, 2 : 5), the solvent was concentrated *in vacuo* and the residue was dissolved in 190 ml of cold THF (0°C). 9.7 ml of 1N HCl were added and the reaction mixture was stirred at 0°C for 15 min until TLC (ethyl acetate/n-hexane, 2 : 5) showed the reaction to be complete. 500 ml of water was added and the mixture was extracted twice with 500 ml of dichloromethane. The dichloromethane layers were combined, washed with brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was stirred overnight in dry diethyl ether (200 ml) and the yellow solid was filtered off. The filtrate was evaporated in vacuo and the remaining solid was purified by flash chromatography (ethyl acetate/n-hexane, 2 : 5) to give a total of 22.2 g (75%) of **42**. m.p. 154-158°C; MS, m/e (EI) 410, 382, 354, 326; ^{1}H-NMR (90 MHz, $CDCl_3$, mixture of isomers, ratio 1 : 1): δ = 0.68 and 0.71 ppm (9H, 2 x s, $C(CH_3)_3$), δ = 1.38-1.72 ppm (4H, m, CH_2-CH_2), δ = 2.23-2.62 ppm (3H, m, H_{10}(ax) + H_8(eq) + H_8(ax)), δ = 3.30-3.66 ppm (3H, m, H_{10a}+ H_{6a} + H_{10}(eq)), δ = 4.38-4.50 ppm (1H, m, H_7), δ = 4.53-4.69 ppm (2H, m, H_1 + H_4), δ = 6.48 ppm (1H, d, J=3.5 Hz, H-C=C), δ = 6.51 ppm (1H, d, J=3.5 Hz, H-C=C), δ = 11.89 and 11.94 ppm (1H, 2 x s, ArOH), δ = 12.34 and 12.35 ppm (1H, 2 x s, ArOH). Anal. calcd. for $C_{24}H_{26}O_6$: C, 70.23; H, 6.38. Found: C, 70.45; H, 6.48.

Cis-(+/-)-7-tert-butoxy-1,4,7,8,9,10-hexahydro-5,9,12-trihydroxy-9-[trimethylsilylethynyl]-1,4-ethanonaphthacene-6,11-dione (44).

To a solution of 4.2 g (0.043 mol) of trimethylsilylacetylene in 450 ml of dry (distilled from sodium benzophenone ketyl) THF at -78°C under an argon atmosphere were added 26.2 ml of 1.6 M n-BuLi in hexane (0.042 mol). After stirring the reaction mixture at -78°C for 30 min, 3.1 g (0.0076 mol) of **42** were added. The reaction was monitored by TLC (ethyl acetate/n-hexane, 2 : 5). After 3 h the reaction mixture was allowed to return to room temperature and 150 ml of 10% aqueous solution of NH_4Cl were added. The mixture was stirred at room temperature for 15 min and 300 ml of water were added. The mixture was extracted twice with 300 ml of chloroform and the organic layers were combined, washed with brine, dried over anhydrous sodium sulfate and then concentrated *in vacuo*. The residue was dissolved in 60 ml of glacial acetic acid and 3.4 g (0.0077 mol) of lead tetraacetate were added to the solution. After stirring the reaction mixture overnight, 200 ml of water were added and the red solid which precipitated was dissolved in 300 ml of chloroform. The organic layer was washed successively with a saturated solution of $NaHCO_3$, water and brine and dried over anhydrous sodium sulfate. The solution was evaporated *in vacuo* and the residue was purified by column chromatography (ethyl acetate/n-hexane, 1 : 4) to give 2.9 g (76%) of red solid **44**. m.p. 116-118°C; ^{1}H-NMR (90 MHz, $CDCl_3$, Mixture of isomers): δ = 0.27 ppm (9H, s, $Si(CH_3)_3$), δ = 1.40 ppm (9H, s, $C(CH_3)_3$), δ = 1.20-1.80 ppm (4H, m, CH_2-CH_2), δ = 1.94 ppm (1H, dd, J=14.5 Hz and J=3 Hz, H_8(ax)), δ = 2.67 ppm (1H, d, J=14.5 Hz, H_8(eq)), δ = 3.04 ppm (1H, d, J=17.5 Hz, H_{10}(ax)), δ = 3.55 ppm (1H, d, J=17.5 Hz, H_{10}(eq)), δ = 4.63 ppm (2H, m, H_1 + H_4), δ = 5.27 ppm (1H, m, H_7), δ = 5.72 ppm (1H, s, OH), δ = 6.48 ppm (2H, dd, H-C=C-H), δ = 12.95 ppm (1H, s, ArOH), δ = 13.12 ppm (1H, s, ArOH). The compound was not obtained completely pure due to some elimination of the trimethylsilyl group. It was used without further purification in the following step.

Cis-(+/-)-7-tert-butoxy-7,8,9,10-tetrahydro-6,9,11-trihydroxy-9-[trimethylsilylethynyl]-5,12-naphthacenedione (45).

A solution of 2.9 g (0.006 mol) of **44** in 30 ml of *o*-xylene was refluxed for 5 h The solution was evaporated *in vacuo* and the solid residue was recrystallized in dry diethyl ether to give 2.5 g (91%) of **45**. m.p. 214-216°C; MS, m/e (EI) 478, 463, 422, 404, 389; ^{1}H-NMR (90 MHz, $CDCl_3$): δ = 0.18 ppm (9H, s, $Si(CH_3)_3$), δ = 1.40 ppm (9H, s, $C(CH_3)_3$), δ = 1.99 ppm (1H, dd, J=14.5 Hz and J=3 Hz, H_8(ax)), δ = 2.76 ppm (1H, d, J=14.5 Hz with long-range coupling, H_8(eq)), δ = 3.02 ppm (1H, d, J=19.5 Hz, H_{10}(ax)), δ = 3.67 ppm (1H, dd, J=19.5 Hz and J=1.5 Hz, H_{10}(eq)), δ = 5.31-5.39 ppm (1H, m, H_7), δ = 5.87 ppm (1H, s, OH), δ = 7.72-7.87 ppm (2H,

m, ArH), δ = 8.25-8.39 ppm (2H, m, ArH), δ = 13.33 ppm (1H, s, ArOH), δ = 13.67 ppm (1H, s, ArOH). Anal. calcd. for $C_{27}H_{30}O_6Si$: C, 67.76; H, 6.32. Found: C, 67.53; H, 6.28.

Cis-(+/-)-7-tert-butoxy-7,8,9,10-tetrahydro-9-acetyl-6,9,11-trihydroxy-5,12-naphthacenedione (46).

To a solution of 2.5 g (0.005 mol) of **45** in 110 ml of THF were added 1.2 g (0.005 mol) of HgO and 55 ml of 3M H_2SO_4. The reaction mixture was stirred for 24 h at room temperature, then poured into 270 ml of 1N HCl and extracted three times with 200 ml of chloroform. After drying over anhydrous sodium sulfate and removal of the solvent *in vacuo*, the solid was purified by column chromatography (ethyl acetate/n-hexane, 2 : 5) to give 1.6 g (91%) of **46**. m.p. 217-220°C; MS, m/e (EI) 424, 368, 350, 332, 307; ^{1}H-NMR (90 MHz, $CDCl_3$) : δ = 1.39 ppm (9H, s, $C(CH_3)_3$), δ = 1.88 ppm (1H, dd, J=14.5 Hz and J=3 Hz, H_8(ax)), δ = 2.37 ppm (1H, d, J=14.5 Hz, H_8(eq)), δ = 2.42 ppm (3H, s, CH_3), δ = 3.04 ppm (1H, d, J_{gem}=19 Hz, H_{10}(ax)), δ = 3.25 ppm (1H, d, J_{gem}=19 Hz, H_{10}(eq)), δ = 5.35-5.44 ppm (1H, m, H_7), δ = 5.94 ppm (1H, s, OH), δ = 7.71-7.84 ppm (2H, m, ArH), δ = 8.21-8.33 ppm (2H, m, ArH), δ = 13.26 ppm (1H, s, ArOH), δ = 13.61 ppm (1H, s, ArOH). Anal. calcd. for $C_{24}H_{24}O_7$: C, 67.92; H, 5.70. Found: C, 68.19; H, 5.65.

(+/-)-4-demethoxydaunomycinone (47).

1.6 g (0.004 mol) of **46** were dissolved in 20 ml of trifluoroacetic acid. After 10 min, 50 ml of water were added and the water layer was then extracted twice with 75 ml of dichloromethane. The combined organic layers were washed with water and brine, then dried and evaporated *in vacuo*. Crude **47** was recrystallized from chloroform/diethyl ether to give 1.2 g (85%) of pure 4-demethoxydaunomycinone. m.p. 183-184°C (Lit.[17] 182.5-183°C); MS, m/e (EI) 368, 350, 332, 307, 290, 279; ^{1}H-NMR (90 MHz, $CDCl_3$) : δ = 2.16 ppm (1H, dd, J=14 Hz and J=5 Hz, H_8(ax)), δ = 2.36 ppm (1H, dt, J=14 Hz, J=2 Hz and J=2 Hz, H_8(eq)), δ = 2.44 ppm (3H, s, CH_3), δ = 2.91 ppm (1H, d, J=18.5 Hz, H_{10}(ax)), δ = 3.23 ppm (1H, dd, J=18.5 Hz and J=2 Hz, H_{10}(eq)), δ = 3.80 ppm (1H, d, J=5.5 Hz, 7-OH), δ = 4.54 ppm (1H, s, OH), δ = 5.30 ppm (1H, brs, H_7), δ = 7.76-7.88 ppm (2H, m, ArH), δ = 8.26-8.40 ppm (2H, m, ArH), δ = 13.25 ppm (1H, s, ArOH), δ = 13.53 ppm (1H, s, ArOH). ^{1}H-NMR data are in full agreement with those described[17].

2.6 References and Notes.

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12 By using the Grignard reagent, only 6% of the trans *tert*-butoxy, hydroxy compound **9** is formed[8]. In similar examples, Potman[8] improved both upscaling and stereoselectivity by using of the *tert*-butoxy group in combination with ethynyllithium. In that case, only a trace of the trans-8,10-hydroxy, *tert*-butoxy compound was formed.

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CHAPTER 3

ABC + D APPROACH TO DAUNOMYCINONE AND DERIVATIVES.

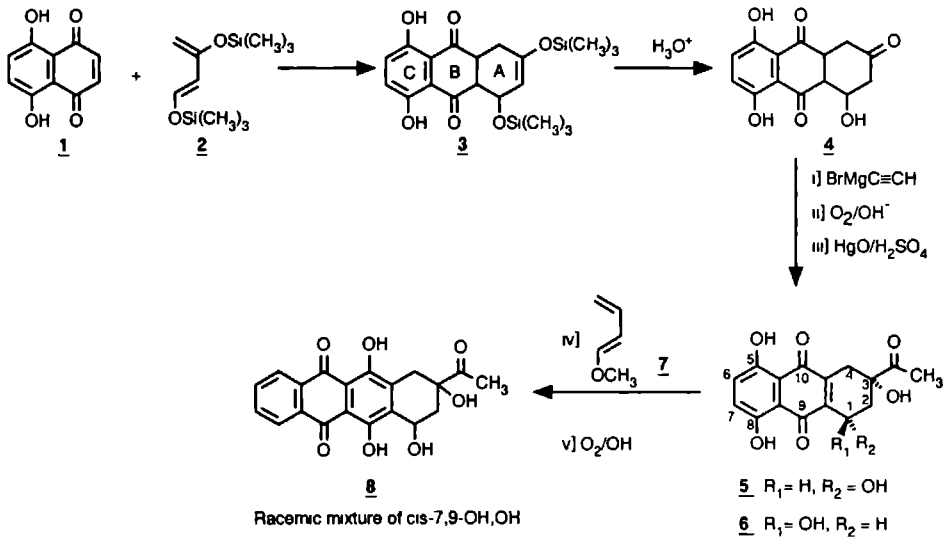
3.1 Introduction.

In the ABC + D approach to daunomycinone and its derivatives, as outlined in Chapter 1, the introduction of the D ring via a Diels-Alder reaction is the key step in the last part of the synthesis. Mixtures of regioisomers are always obtained when the method is used for the synthesis of unsymmetrically substituted D ring derivatives, as there is no control over regioselectivity in the Diels-Alder addition. Based on the method of Krohn *et al.*[1], we have developed a practical gram-scale approach which allows the regioselective synthesis of daunomycin and some derivatives.

3.2 Synthesis of the ABC fragment.

Krohn and Tolkiehn[1] have published a method in which naphthazarin 1 and 1,3-bis-(trimethylsilyloxy)-buta-1,3-diene 2 were used for the synthesis of the ABC fragment 3 of daunomycinone (Scheme 3.1).

Scheme 3.1



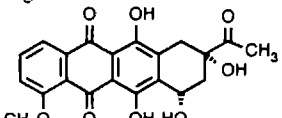
Racemic mixture of cis-7,9-OH,OH

After hydrolysis, treatment with 30 equiv. of ethynylmagnesium bromide, oxidation and hydration with mercury(II) oxide, the ABC fragment 5 was used in another Diels-Alder reaction with 1-methoxybuta-1,3-diene 7 to give rise to 4-demethoxydaunomycinone 8 (Scheme 3.1).

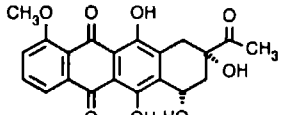
Separation of the ABC fragments 5 and 6 (Scheme 3.1) was very difficult and was only

accomplished after preparative thin-layer chromatography. The Diels-Alder reaction of 1-methoxycyclohexa-1,3-diene, instead of 7, with 5, gave after oxidation and retro Diels-Alder reaction, a mixture of daunomycinone 9 and isodaunomycinone 10 in yields of 17% and 32%, respectively (Figure 3.1).

Figure 3 1



9 (Rac mixt of cis-7,9 OH,OH)

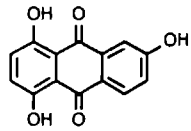


10 (Rac. mixt of cis-7,9-OH,OH)

Repeating the reaction sequence of Scheme 3.1 in our laboratory showed more disadvantages than just the lack of regioselectivity already described by Potman[2].

- One of these major disadvantages is aromatization of compound 4. The hydroxy group of compound 4 easily undergoes elimination and, when followed by enolisation and spontaneous air oxidation, the aromatic compound 11 is formed (Figure 3.2).

Figure 3 2

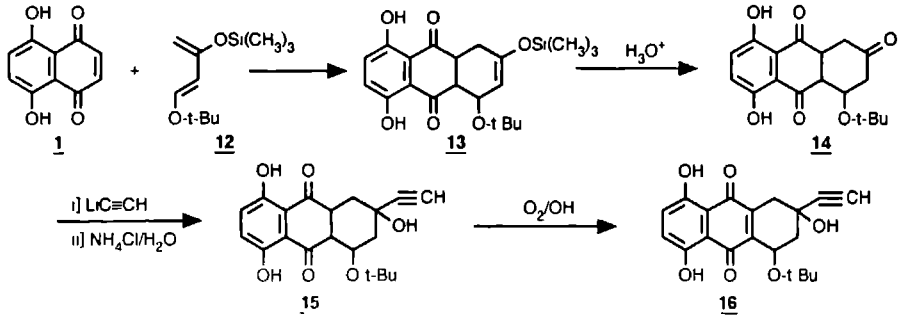


11

- Compounds 5 and 6 have very low solubilities, which makes it necessary to use large amount of solvent for the subsequent conversion step.
- The requirement for an excess of Grignard reagent (30 equivalents) limits the practical value of this synthetic approach and makes it unsuitable for multigram syntheses.
- Formation of both cis-(5)- and trans-(6)-1,3-di-hydroxy isomers (Scheme 3.1), after the Grignard reaction, made it necessary to separate those isomers after hydrolysis with HgO and H_2SO_4 in order to isolate the desired (+/-)-demethoxydaunomycinone 8.

Potman[2] has employed the 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene 12 (Scheme 3.2) in order to reduce the aromatization of product 14 and to improve the solubility of the product 16.

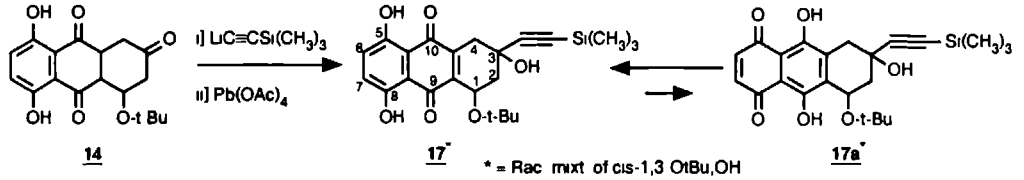
Scheme 3.2



Instead of the bromomagnesium acetylide, he used ethynyllithium, which was needed only in five-fold excess. Due to the bulky *tert*-butoxy group, the cis-1-*tert*-butoxy, 3-hydroxy-isomer **15** was formed predominantly, while only a trace of the trans isomer of compound **15** was detected by NMR spectroscopy. We encountered some problems in the synthesis of the ABC fragment on a multigram scale. The decrease of yield with increasing scale of the reaction was mainly ascribed to increasing aromatization of **14**, leading to **11** (Figure 3.2). For practical reasons the concentration of Li-C≡C-H was increased for large scale reactions. This higher concentration of Li-C≡C-H leads to higher amount of trimeric (or even polymeric) organolithium compounds, which do not give addition to the carbonyl group, but which can act as strong bases, causing aromatization. Using of for example, Li-CH(OCH_3)=CH_2, as acyl equivalent, we observed the same problems.

We expect that the bulky trimethylsilyl group of 2-trimethylsilylethynyllithium prevents formation of trimers and polymers from the organolithium compounds. By using this 2-trimethylsilylethynyllithium, easily prepared from the commercially available 2-trimethylsilylacetylene and n-butyllithium, we were able to synthesize compound **17** on a 2.5 g scale (Scheme 3.3).

Scheme 3.3

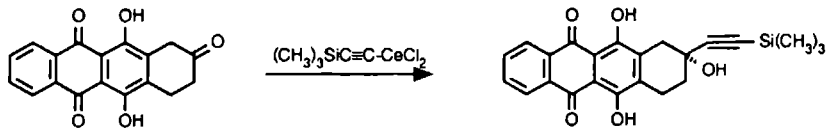


Although a low concentration of the 2-trimethylsilylethynyllithium (approximately 0.08 mmol/ml) is still necessary to obtain reasonable yields, further upscaling of this reaction should be possible, at least in principle, because yields at the mg scale were the same as at gram scale.

Another advantage of using the more bulky 2-trimethylsilylethynyllithium is the exclusive formation of the cis-1-*tert*-butoxy-3-hydroxy compound **17**. No trans product was detected by ^{1}H-NMR spectroscopy.

In 1984 Suzuki and Terashima[3] reported the use of 2-trimethylsilylethynylcerium(III) dichloride as an alternative for the bromomagnesiumacetylide. The cerium reagent was formed by the addition of dry $CeCl_3$ to 2-trimethylsilylethynyllithium. The examples described all had no functionality at the 1 position (Scheme 3.4). We have also tried to use 2-trimethylsilylethynylcerium(III) dichloride in the reaction with triketone **14**. However, no advantages for this cerium reagent were found. Yields were not improved and the experimental conditions were more complex (especially the preparation of the cerium reagent).

Scheme 3.4



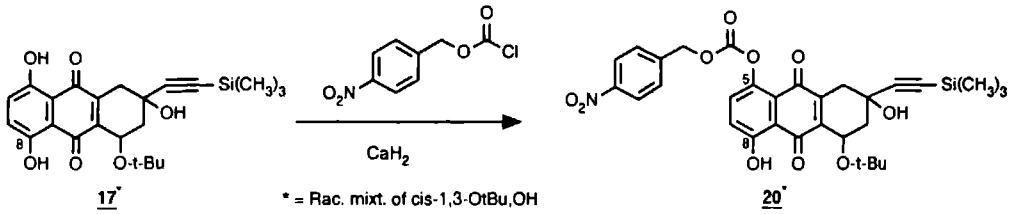
18 19

3.3 Synthesis of daunomycinone.

The practical multigram scale approach to **17** opens attractive routes to the synthesis of 'symmetrically' substituted D-ring derivatives but the lack of regioselective control must still be solved.

As described earlier, Krohn and Tolkiehn[1] isolated only 17% of daunomycinone **9**, while 32% of isodaunomycinone **10** was formed (Figure 3.1). The regioselectivity of the cycloaddition used is not appropriate with regard to formation of the desired daunomycinone. Therefore, we tried to increase the regioselectivity of the cycloaddition by protecting of one of the aromatic hydroxy groups of compound **17**. In Chapter 2, we described selective protection of the DCB fragment with p-nitrobenzyl chloroformate. This protected DCB fragment reacted regioselectively with 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene. Therefore, we tried to apply the same p-nitrocarbobenzoxy group for the protection of the ABC fragment **17** in order to increase the regioselectivity of the subsequent Diels-Alder reaction (Scheme 3.6).
We expected that the bulky *tert*-butoxy group in **17** would shield the hydroxy group at the 8 position. Indeed, reaction of **17** with 1.1 equivalent of p-nitrobenzyl chloroformate yielded one predominant product, namely regioisomer **20** (Scheme 3.6).

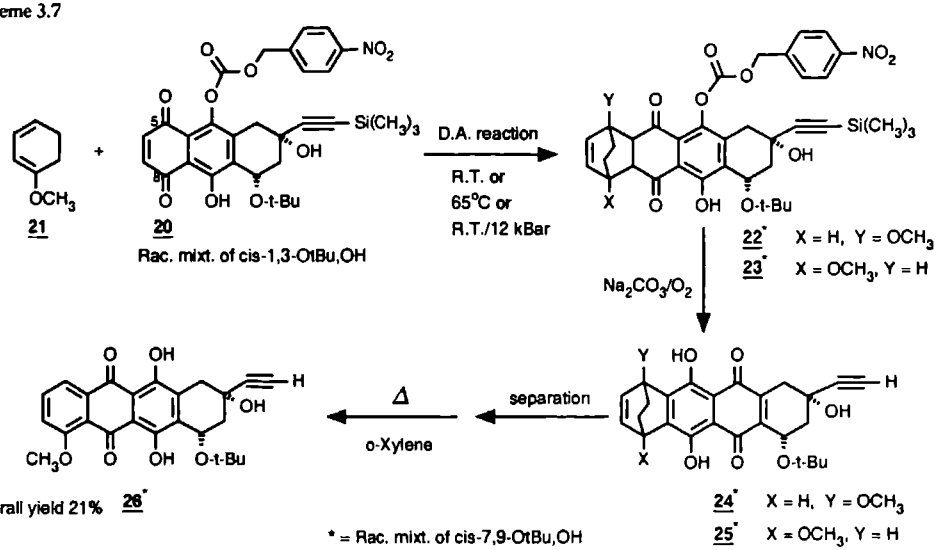
Scheme 3.6



17* * = Rac. mixt. of cis-1,3-OtBu,OH 20*

After column chromatography, 70% of **20** was isolated and only small amounts of the other mono protected compound (8 position) and of the diprotected compound (5 and 8 positions) were found, together with a small amount of unreacted **17**.
The Diels-Alder reaction of **20** with 1-methoxycyclohexa-1,3-diene **21** (Scheme 3.7) was rather slow. After stirring a solution of **17** and 1-methoxycyclohexa-1,3-diene **21** in chloroform at room temperature for 7 days no more than 50% of **20** was converted into the Diels-Alder adducts **22**/**23** (Scheme 3.7).

Scheme 3.7



At 65°C, the reaction was considerably accelerated (2 days, 95% conversion). High pressure (12 kbar) also accelerated the Diels-Alder reaction (70%, 16 h, 25°C, chloroform). Regioselectivity was not complete since ^{1}H-NMR showed that two products were formed. The main product (>90%) was 22 with the methoxy group and the *tert*-butoxy group on the same side of the molecule. Oxidation of 22/23 with OH^-/O_2, as described by Krohn and Tolkiehn[1], was not possible. Thin-layer chromatography showed that fast deprotection occurred, but oxidation of the bond between the C and D rings was rapidly followed by aromatization of the A ring. With 5% sodium carbonate, complete deprotection was achieved (although not so rapidly). Oxidation was very slow but aromatization did not occur. The reaction was monitored by thin-layer chromatography and the reaction was stopped after all deprotected 22/23 had disappeared. A mixture of 24/25 was obtained, which was separated into the two components by column chromatography. Due to the long exposure (24 h) to Na_2CO_3, deprotection of the ethynyl function had also occurred.

The regioselectivity of the above reactions was determined by comparison of the ^{1}H-NMR spectra of reaction mixture of 24/25 (Scheme 3.7) with the previously prepared compound (Chapter 2, 27). The position of the aromatic hydroxy groups is very characteristic. The largest shift difference between the two hydroxy protons is found when the methoxy group and the *tert*-butoxy group are on the same side of the molecule.

Retro Diels-Alder reaction of 25 yielded 26 (Scheme 3.7), which was isolated pure after crystallization from diethyl ether (overall yield of 21% starting from 20). This product was further converted into daunomycinone in the same way as compound 27 (Chapter 2).

3.4 Conclusions

In this chapter, the synthesis of daunomycinone according to the ABC + D approach has been worked out. By modifying essential steps in the method of Krohn and Kolkiehn[1] the ABC fragment was synthesized on a gram scale. The use of 1-*tert*-butoxy-3-trimethylsilyloxy-1,3-butadiene (**12**) made the Diels-Alder adduct more soluble and, together with the use of 2-trimethylsilylethynyllithium, this resulted in exclusive formation of the cis-1-*tert*-butoxy-3-hydroxy compound **17**. By regioselective protection of the hydroxy group of **17** at the 5 position (Scheme 3.6) with a p-nitrocarbobenzoxy group, the regioselectivity in the subsequent Diels-Alder reaction was strongly increased, changing from 1:2 (compound **9** : **10**, Figure 3.1) to 9:1 (compound **24** : **25**, Scheme 3.7). Although daunomycinone was synthesized in this way, the method described in Chapter 2 is preferred, as in this case no separation of regioisomers was necessary. This method is however, a good way to prepare D ring derivatives of daunomycine, as described in Chapter 6, in which the total synthesis of pyridine D ring derivatives of daunomycin by this method is described.

3.5 Experimental section.

General remarks:
^{1}H-NMR spectra were measured on a Bruker WH-90, a Bruker AM-100 or a Bruker AM-400 spectrometer using Me_4Si as internal standard. $CDCl_3$ was used as solvent. Melting points were taken with a Reichert Thermpan microscope and are uncorrected. Mass spectra were taken using a double focusing VG 7070 E mass spectrometer. For column chromatography, either Merck Silicagel 60 or Merck silicagel Art. 9385 (flash chromatography) were used.

*1-tert-Butoxy-3,4,4a,9a-tetrahydro-5,8-dihydroxy-3,9,10(2H)-anthracenetrione (**14**).*

To a solution of 9.0 g (0.047 mol) of 5,8-dihydroxy-1,4-naphthoquinone **1** in 175 ml of dry THF (distilled from sodium benzophenone ketyl) under an argon atmosphere, 14.2 g (0.066 mol) of 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene **2** were added. Progress of the reaction was monitored by TLC (ethyl acetate/n-hexane, 2:5). After 1 h, the reaction mixture was cooled to 0°C and 5.75 ml of 1N HCl were added. After 10 min, 125 ml of water were added and the reaction mixture was extracted twice with 225 ml of chloroform. The combined organic layers were then washed with 125 ml of water, 125 ml of brine and dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the remaining solid was rinsed overnight with 300 ml of diethyl ether. After cooling the solution to 0°C a light yellow solid was collected by filtration and washed with cold diethyl ether. The yield was 12.1 g (78%). m.p. 180°C. (Lit[2] 180°C); ^{1}H-NMR (90 MHz, $CDCl_3$) : δ = 0.75 ppm (9H, s, $C(CH_3)_3$), δ = 2.37 ppm (1H, dd, J=15.6 Hz and J=7.5 Hz, CH_2), δ = 2.58 ppm (2H, d, J=2.5 Hz, CH_2), δ = 3.33-3.73 ppm (3H, m), δ = 4.42-4.50 ppm (1H, m, H_1), δ = 7.25 ppm (1H, AB, J=8.5 Hz, ArH), δ = 7.31 ppm (1H, AB, J= 8.5 Hz, ArH), δ = 12.10 ppm (1H, s, ArOH), δ = 12.30 ppm (1H, s, ArOH).

*1-tert-Butoxy-1,2,3,4-tetrahydro-3,5,8-trihydroxy-3-(2-trimethylsilylethynyl)-9,10-anthracenedione (**17**).*

To a solution of 6.0 g (0.061 mol) of trimethylsilylacetylene in 740 ml of dry THF (distilled from sodium benzophenone ketyl) at -78°C under an argon atmosphere were added 24.1 ml of 2.45M n-BuLi in n-hexane (0.060 mol). After stirring the reaction mixture at -78°C for 30 min 4.0 g (0.012 mol) of **14** were added. The reaction was monitored with TLC (ethyl acetate/n-hexane, 2:5). After 3 h, the reaction mixture was allowed to return to room temperature and 150 ml of a 10% aqueous solution of NH_4Cl were then added. The mixture was

stirred at room temperature for 15 min and then 300 ml of water were added. The mixture was extracted twice with 300 ml of chloroform and the organic layers were combined, washed with brine and evaporated *in vacuo*. The residue was dissolved in 100 ml of glacial acetic acid and 5.3 g (0.012 mol) of lead tetraacetate were then added to the solution. After stirring the reaction mixture overnight, 200 ml of water were added and the red solid which precipitated was dissolved in 300 ml of chloroform. The organic layer was washed successively with a saturated solution of $NaHCO_3$, water and brine and dried over anhydrous sodium sulfate. The solution was evaporated *in vacuo* and rinsed overnight in 100 ml of dry diethyl ether to give 2.6 g (50%) of a red solid **17**. m.p. 191-193°C; ^{1}H-NMR (90 MHz, $CDCl_3$): δ = 0.18 ppm (9H, s, $Si(CH_3)_3$), δ = 1.33 ppm (9H, s, $C(CH_3)_3$), δ = 1.87 ppm (1H, dd, J=3.7 Hz and J=14.5 Hz, H_2(ax)), δ = 2.64 ppm (1H, d, J=14.5 Hz, H_2(eq)), δ = 2.77 ppm (1H, d, J=20 Hz, H_4(ax)), δ = 3.47 ppm (1H, d, J=20 Hz, H_4(eq)), δ = 5.19 ppm (1H, m, H_1), δ = 5.57 ppm (1H, s, OH), δ = 7.18 ppm (2H, s, ArH), δ = 12.56 ppm (1H, s, ArOH), δ = 12.69 ppm (1H, s, ArOH). Anal. calcd. for $C_{23}H_{28}O_6Si$: C, 64.46; H, 6.59. Found: C, 64.94; H, 6.61.

1-tert-Butoxy-1,2,3,4-tetrahydro-3,8-dihydroxy-5-(4-nitrobenzyloxycarbonyloxy)-3-(2-trimethylsilylethynyl)-9,10-dioxoanthracene (**20**).

To a solution of 5.0 g (0.012 mol) of **17** and 3.0 g (0.014 mol) of 4-nitrobenzyloxycarbonylchloride in 150 ml of dry THF (distilled from sodium benzophenone ketyl), 0.5 g (0.012 mol) of powdered sodium hydride were added. The suspension was sonicated with a Direct Immersion Sonic Horn[4] (Cell disrupter) in a glass rosette cell[4] at 0°C for 4 h and then the reaction mixture was poured into a 250 ml round-bottom flask and sonicated for 24 h in an ultrasonic cleaning bath at 60°C. The color of the solution changed from red to yellow and progress was monitored by TLC (ethyl acetate/ n-hexane, 2 : 5). After TLC indicated that the reaction was nearly complete, the reaction mixture was quenched by addition of 500 ml of 5% aqueous solution of NaH_2PO_4 and the mixture was then extracted twice with 500 ml of chloroform. The chloroform extract was washed with brine, dried over anhydrous sodium sulfate and then evaporated *in vacuo*. The crude product was purified by column chromatography (ethyl acetate/n-hexane, 1 : 4). The remaining solid residue after evaporation *in vacuo* was stirred overnight in 50 ml of diisopropyl ether. Filtration gave 5.0 g (71%) of pure **20**. m.p. 164°C; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS as internal standard) : δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 1.36 ppm (9H, s, $C(CH_3)_3$), δ = 1.90 ppm (1H, dd, J=3.75 Hz and J=14.5 Hz, H_2(ax)), δ = 2.67 ppm (1H, d, J=14.5 Hz, H_2(eq)), δ = 2.77 ppm (1H, d, J=20 Hz, H_4(ax)), δ = 3.47 ppm (1H, d, J=20 Hz, H_4(eq)), δ = 5.21 ppm (1H, s, H_1), δ = 5.43 ppm (2H, s, CH_2), δ = 5.54 ppm (1H, s, 3-OH), δ = 7.33 ppm (2H, dd, H_6+H_7), δ = 7.69 ppm (2H, AB, J=8.6 Hz, ArH), δ = 8.29 ppm (2H, AB, J= 8.6 Hz, ArH), δ = 12.47 ppm (1H, s, ArOH). Anal. calcd. for $C_{31}H_{33}NO_{10}Si$: C, 61.27; H, 5.47; N, 2.30. Found: C, 61.27; H, 5.54; N, 2.38.

Cis-(+/-)-7-tert-butoxy-1,4,7,8,9,10-hexahydro-9-ethynyl-5,9,12-trihydroxy-1,4-ethanonaphthacene-6,11-dione (**25**).

1.5 g of 1-Methoxy-1,3-cyclohexadiene **21** (0.014 mol, 3.3 equivalents) were added to a solution of 2.5 g (0.004 mol) of **20** in 75 ml of chloroform and stirred at 65°C for 35 hours. After evaporation *in vacuo* the residue was dissolved in 200 ml of THF and 400 ml 5% aqueous solution of Na_2CO_3 were added while air was bubbled through the solution. After TLC showed no more starting material (24 h), the reaction mixture was neutralized with acetic acid until its color turned red. The water layer was then extracted three times with 250 ml of chloroform. The combined organic layers were washed with 250 ml of a solution of saturated sodium hydrogen carbonate, with 250 ml of water and 250 ml of brine. After drying over anhydrous sodium sulfate and concentration under reduced pressure, the residue was purified by column chromatography to give 0.8 g (42%) of pure **25**. m.p. 156-158°C (dec.); ^{1}H-NMR (100 MHz): δ = 1.35 ppm (9H, s, $C(CH_3)_3$), δ = 1.20-1.98 ppm (5H, m, CH_2-CH_2 and H_2(ax)), δ = 2.33 ppm (1H, s, C≡CH), δ = 2.69 ppm (1H, d, J=14.6 Hz, H_9eq)), δ = 2.81 ppm (1H, d, J=20 Hz, H_7ax)), δ = 3.47 ppm (1H, d, J=20 Hz, H_7(eq)), δ = 3.74 ppm (3H, s, OMe), δ = 4.55 ppm (1H, d, J=6 Hz, H_4), δ = 5.23 ppm (1H, s, H_{10}), δ = 5.77 ppm (1H, s, 8-OH), δ = 6.45 ppm (1H, dd, J=8 Hz and J=6 Hz, H_3), δ = 6.70 ppm (1H, d, J=8 Hz, H_2), δ = 12.84 ppm (1H, s, ArOH), δ = 13.64 ppm (1H, s, ArOH).

Cis-(+/-)-10-tert-butoxy-7,8,9,10-tetrahydro-8-ethynyl-1-methoxy-5,8,12-trihydroxynaphthacene-6,11-dione (26).

A solution of 0.8 g (0.0017 mol) of 25 in 100 ml of *o*-xylene was refluxed for 30 minutes. After evaporation *in vacuo* and tituration with diethyl ether, 0.45 g of orange solid was isolated which was recrystallized in ethyl acetate/n-hexane to give 0.38 g (51%) of 26. m.p. 260-262°C; ^{1}H-NMR (100 MHz): δ = 1.39 ppm (9H, s, $C(CH_3)_3$), δ = 2.03 ppm (1H, dd, J=14.5 Hz and J=2.5 Hz, H_2(ax)), δ = 2.56 ppm (1H, s, C≡CH), δ = 2.77 ppm (1H, d, J=14.5 Hz, H_9(eq)), δ = 2.96 ppm (1H, d, J=19.7 Hz, H_7(ax)), δ = 3.65 ppm (1H, d, J=19.7 Hz, H_7(eq)), δ = 4.07 ppm (3H, s, OMe), δ = 4.55 ppm (1H, d, J=6 Hz, H_4), δ = 5.37 ppm (1H, brs, H_{10}), δ = 6.03 ppm (1H, s, 8-OH), δ = 7.35 ppm (1H, d, J=8 Hz, H_3), δ = 7.74 ppm (1H, dd, J=8 Hz and J=7 Hz, H_3), δ = 7.99 ppm (1H, d, J=7 Hz, H_2), δ = 13.28 ppm (1H, s, ArOH), δ = 14.08 ppm (1H, s, ArOH).

3.6 References.

1 Krohn, K. and Tolkiehn, K., *Chem. Ber.*, **1979**, *112*, 3453.
2 Potman, R.P., Ph.D. Thesis, Nijmegen, Chapter 5.
3 Suzuki, M. and Terashima, S., *Chem. Lett.*, **1984**, 1543.
4 Lindley, J. and Mason, T.J., *Chem. Soc. Rev.*, **1987**, *16*, 275.

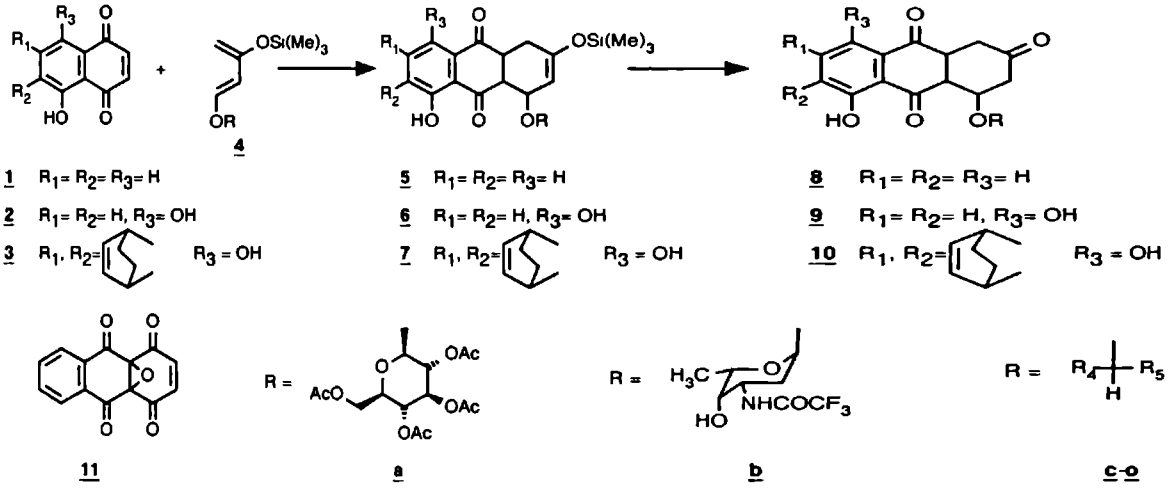
CHAPTER 4

HIGH DIASTEREOMERIC INDUCTION IN DIELS-ALDER REACTIONS OF QUINONES WITH CHIRAL 1-OXY-3-TRIMETHYLSILYLOXYBUTA-1,3-DIENES HAVING SUBSTITUENTS WITH π OR p ELECTRONS IN APPROPRIATE POSITIONS.

4.1 Introduction.

In several syntheses of daunomycinone and D ring substituted analogs, as described in this thesis and elsewhere[1-4], the Diels-Alder reaction of a quinone precursor with 1-alkoxy-3-trimethylsilyloxybuta-1,3-diene is a key step (Scheme 4.1).

Scheme 4.1

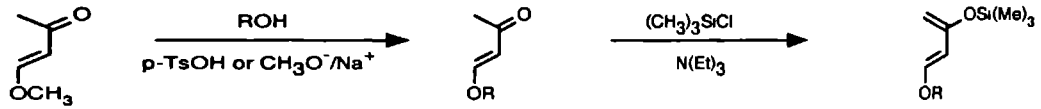


In our laboratory, diastereoselective control of this reaction has been studied earlier by Potman[4] with dienes (4) having chiral auxiliary groups R, such as menthyl or bornyl. A maximum diastereomeric excess (d.e.) of 30% was established in the cycloaddition of 4 (R = bornyl) with naphthazarin (2). Stoodley and colleagues[5] and Brasca and Penco[6] studied 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside 4a and amino protected-α-D-daunosamine 4b, respectively, as chiral alkoxy groups in the cycloaddition reaction with the highly reactive dienophile quinizarine quinone epoxide 11. In our hands, diene 4a appeared to be not sufficiently reactive to give satisfactory conversions with less reactive quinones such as naphthazarin 2[1,7,8] and 1,4,9,10-tetrahydro-5,8,-dihydroxy-9,10-dioxo-1,4- ethanoanthracene 3 used in our[3] (Chapter 2) and other approaches[2]. Therefore, we have studied in a systematic way the diastereomeric induction of a series of more electron-rich substituted 1-oxy-3-trimethylsilyloxybuta-1,3-dienes (4) in the cycloaddition reaction with quinones.

4.2 Diels-Alder reaction of 1-alkoxy-3-trimethylsilyloxy-1,3-butadienes with several dienophiles.

We first investigated dienes having secondary alkoxy groups, in the cycloaddition with naphthazarin (2) and juglone (1) (Scheme 4.1). The dienes were synthesized according to the method of Danishefsky *et al.*[9] from racemic alcohols ROH (Scheme 4.2).

Scheme 4.2



12 13 c-r 4 c-r

The formed cycloadducts (5-7) were hydrolysed to the corresponding ketones (8-10). The d.e.[10] value was determined by ^{1}H-NMR spectroscopy[11] and HPLC analysis of both the crude reaction mixture and the isolated cycloadducts. The highest d.e. (30%), when R_5 is an aliphatic group, was measured for entry 3 (9e, Table 4.1). There is an obvious but low steric effect of the size of the R_5 group on the diastereomeric induction.

Table 4.1

Diels-Alder reactions of dienes 4c-m with dienophiles 1 and 2*

Entry	Diene	R_4	R_5	8 d e**	8 yield	9 d e**	9 yield
1	4c	Me	Et	3	72	5	71
2	4d	Me	i Pr	6	74	15	75
3	4e	Me	t-Bu	15	78	30	69
4	4f	Me	cyclohexyl	26	45	26	63
5	4g	Me	Ph	66	79	70	62
6	4h	Et	Ph	60	76	50	67
7	4i	i-Pr	Ph	50	86	30	48
8	4j	Me	CH_2OMe	30	77	35	80
9	4k	Me	$CH{=}CH_2$	45	76	50	62
10	4l	Me	CH_2CH_2OMe	0	63	0	69
11	4m	H	PhCH(Me)	5	88	8	55

* reactions were carried out using racemic dienes 4 in THF (0 1 molar solutions with the dienes in 50% excess) The cycloaddition was complete after 16-20 h at room temperature The yields are isolated yields after precipitation in THF/n hexane

** diastereomeric excess (d e) in % determined by ^{1}H-NMR spectroscopy The d e of the reaction mixture determined using HPLC for the cycloadditions of 2 showed the same ratios as found using ^{1}H NMR

However, the chiral dienes 4 with R_4 = alkyl and R_5 = phenyl (entries 5 - 7) gave unexpectedly high d.e. values (Table 4.1). The highest induction (70%) was found for the cycloaddition of naphthazarin (2) with 1-[1-phenylethoxy]-3-trimethylsilyloxybuta-1,3-diene (entry 5). This is clearly higher than would be expected on the basis of steric differences between R_4 and R_5. This effect was also observed for other alkoxy groups with R_5 having π electrons or non-bonded electron pairs close to the chiral center, such as 2-methoxyisopropoxy (entry 8) and 1-vinylethoxy (entry 9). Increasing the size of the R_4 group for R_5 = phenyl (entries 5 -7) lowers the d.e. Increasing the distance of the chiral center to the methoxy or phenyl substituent in R_5 leads to a large decrease in the d.e. (e.g., entries 10 and 11).

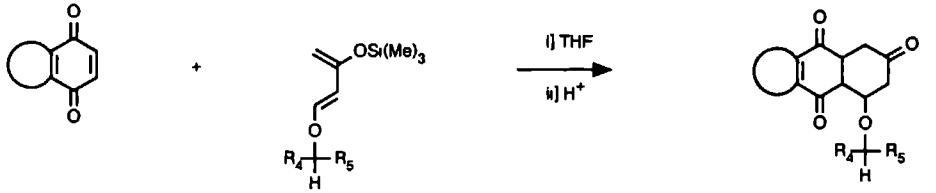
The effect of the electron density of the phenyl group in R_5 on the d.e. is presented in Table 4.2. Juglone (1), naphthoquinone (14) and benzoquinone (15) were studied in addition to naphthazarin (2). For juglone and benzoquinone, as shown in Table 4.2, we found that d.e. decreases with decreasing electron density of the phenyl ring in the chiral group. For naphthazarin, this decrease was also noted, but the values for the para-methyl- and para-chloro-substituted phenyl analogs were not as expected. The d.e. of the reaction with naphthoquinone showed no good correlation with the decreasing electron density of the phenyl ring. In every case however, lowest induction was found with diene 4g having the strongest electron-withdrawing CF_3 substituent (entry 5). The largest d.e. was found in the reaction of benzoquinone (15) with 1-[1-(*p*-methoxyphenyl)-ethoxy]-3-trimethylsilyloxybuta-1,3-diene (entry 12).

4.3 Diels-Alder reaction of chiral 1-(1-phenylethoxy)-3-trimethylsilyloxy-1,3-butadienes with benzoquinone.

In the reaction of benzoquinone (15) with 1-[1-(*p*-methoxyphenyl)-ethoxy]-3-trimethylsilyloxybuta-1,3-diene (4n), only one isomer was detected using ^{1}H-NMR spectroscopy. Because the optically active 1-(*p*-methoxyphenyl)-ethanol was not available, we used S-(-)- and R-(+)-1-phenylethanol to determine the absolute configuration of the Diels-Alder adduct with benzoquinone 15. The d.e. found for this reaction was 90% and we were able to isolate the pure diastereomer after several crystallization steps.

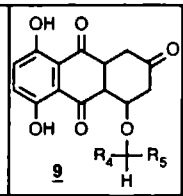
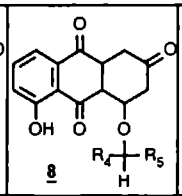
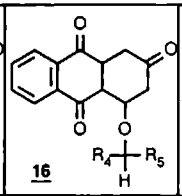
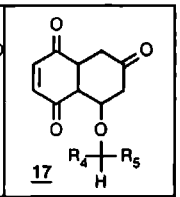
Table 4.2

Diels-Alder reaction of dienes 4 with dienophiles 1, 2, 14 and 15*



1, 2, 14 and 15 | 4 g and n-r | 8, 9, 16 and 17

Table 4 2 continued

				 9		 8		 16		 17	
Entry	Diene	R_4	R_5	d e [**]	yield	d e [**]	yield	d e [**]	yield	d e [**]	yield
12	4n	Me	p-MeO-Ph	92	54	68	40	77	60	99	49
13	4o	Me	p-Me-Ph	64	50	67	55	84	50	97	60
5	4g	Me	Ph	70	62	66	79	90	53	90	53
14	4p	Me	p-Cl-Ph	84	55	62	44	90	19[***]	80	52
15	4q	Me	p-CF_3-Ph	57	51	46	53	64	49	63	55
16	4r	Et	p-CF_3 Ph	56	78	42	78	-	-	-	-

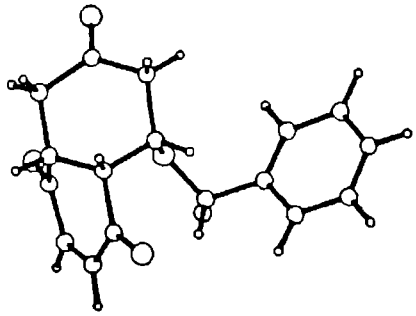
[*] reactions were carried out using racemic dienes 4 in THF (0 1 molar solutions with the diene in 50% excess) The cycloaddition was complete after 16-20 h at room temperature The yields are isolated yields after precipitation in THF/n hexane

[**] diastereomeric excess (d e) in % determined by ^{1}H NMR spectroscopy The d e of the reaction mixture determined using HPLC for the cycloadditions of 4g and n-o with 2 showed the same ratios as found using ^{1}H NMR

[***] Low isolated yield due to problems with precipitation and small quantities, conversion (TLC) almost complete

X-ray analysis (Figure 4.1) of the cycloadduct of benzoquinone 15 and S-(-)-diene 4g showed that the formed chiral center also has the S configuration at the position at which the chiral alkoxy group is attached.

Figure 4 1



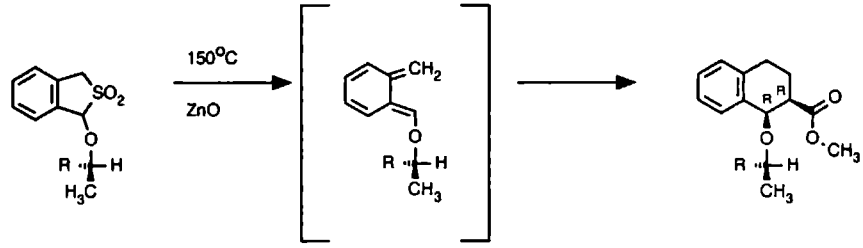
Breitmaier *et al.*[12] who independent from us have studied cycloadditions of quinones with 1-(1-phenylethoxy)-2-methyl-buta-1,3-dienes, explained the found diastereoselectivity using a π stacking model, which had previously been used by Trost *et al.*[13] for related cycloadditions of butadienyl-O-methyl mandelates (Figure 4.2). In this model, overlap of the orbitals of the phenyl ring would lead to two equilibrating complexes in which the phenyl ring is in a nearly parallel plane above or below the plane of the diene.

Figure 4.2

The large (L) and small (S) substituents make conformation B (Figure 4.2) sterically more favored[12]. Diastereoselectivity is obtained by preferred approach of the dienophile to the unshielded diene side. From models, it can be easily seen that in the case of 1-[1-phenyl-ethoxy]-3-trimethylsilyloxybuta-1,3-diene, the diene plane forms a wide angle with the plane of the phenyl ring so that π-π interactions will be weak.

For cycloadditions of *o*-quinodimethanes also having the 1-phenylethoxy group as chiral auxiliary (Scheme 4.3), Charlton *et al.*[14] showed that π stacking could not explain the found diastereoselectivity. He suggested that the preferred conformation for the diene is as in **19** (Scheme 4.3). The approach of the dienophile to **19** is determined by the difference in steric bulkiness of the methyl group and the R group.

Scheme 4.3

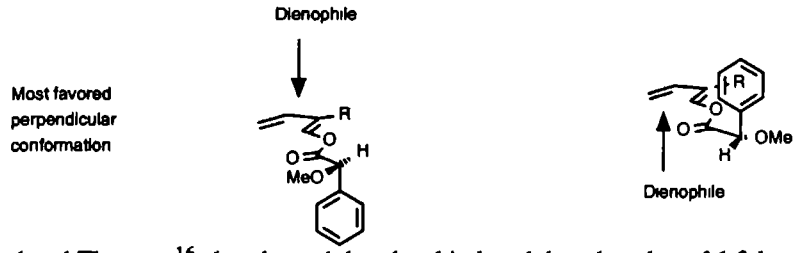


18 **19** **20**

Entry	R =	d.e (%)
a.	Ph	47
b.	t-butyl	16
c.	cyclohexyl	0
d.	$PhCH_2$	0
e.	$PhCH_2CH_2$	0

In a more recent paper, Tucker *et al.*[15] retreated from the π stacking model because of a molecular mechanics study of the Diels-Alder reactions of chiral 1,3-butadienyl-O-methyl-mandelate. Their results support an earlier statement of Siegel and Thornton[16] based on experimental results indicating that the orientation of the phenyl ring is perpendicular to the diene plane. In the most favored perpendicular conformation, the methoxy group is proposed to be preferentially eclipsed with the carbonyl oxygen[15] (Figure 4.3).

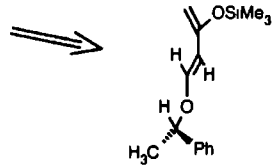
Figure 4.3



Siegel and Thornton[16] also showed that the chiral cyclohexyl analog of 1,3-butadienyl-O-methyl mandelate, which is not capable of π stacking, had almost equal facial selectivity and the same direction of enantioselectivity.

In agreement with the published results discussed above, two models may explain our results. Analogous to the model of Charlton *et al.*[14] (Scheme 4.3), the diene adopts in the transition state (TS), a conformation as in Figure 4.4. The diene is most easily approached from the (less hindered) rear side of the molecule (with the methyl substituent) as shown in Figure 4.4.

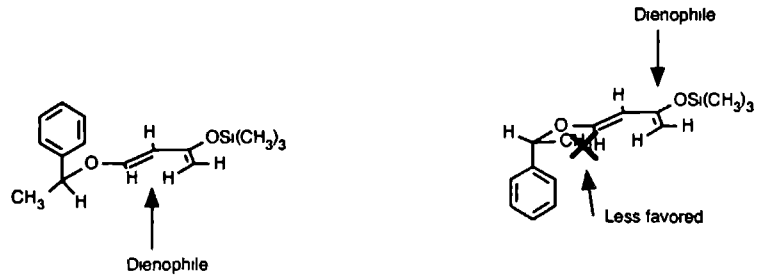
Figure 4.4



The higher induction for R = phenyl over R = *t*-butyl can be ascribed to a larger effective size of the phenyl substituent due to specific and stronger solvation by the used solvent toluene[14]. However, we found practically the same induction for cycloadditions of diene **4g** in the solvents cyclohexane, THF and benzene, which makes this model unlikely. Furthermore, higher induction is expected in the donor solvent THF for dienes having electron-withdrawing phenyl substituents, whereas we found the opposite effect.

The second model, the perpendicular TS model analogous to that proposed by Siegel and Thornton[16] for cycloadditions of 1,3-butadienyl-O-methylmandelates (Figure 4.3), can help to explain all of our results. This model is presented in Figure 4.5.

Figure 4.5



In the sterically favored perpendicular conformation, the quinone will approach the diene in the Diels-Alder reaction from a side opposite to the aryl group, for steric reasons, and also to avoid electrostatic repulsion between the electron-rich phenyl ring and one of the oxygen atoms of the quinone. For that reason, induction is affected by substituents on the phenyl ring, leading to higher d.e. in the case of donating substituents (Table 4.2)

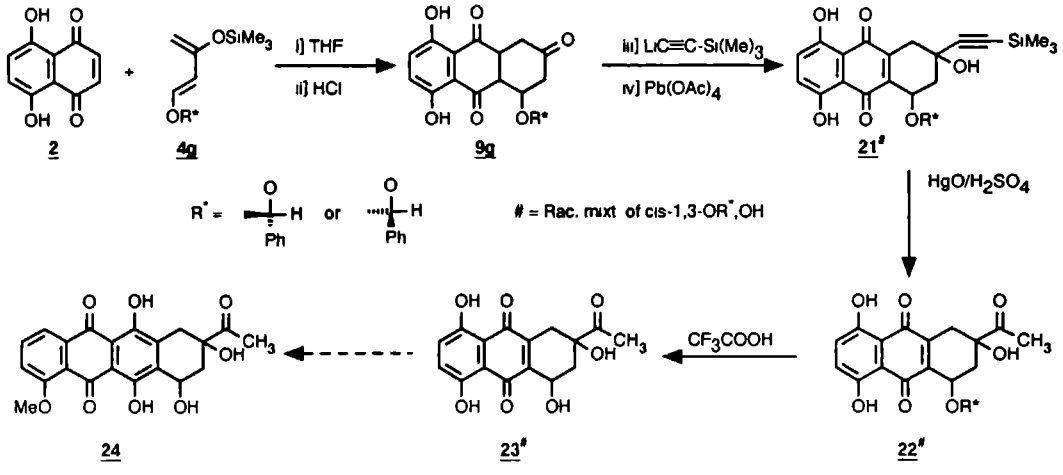
Other substituents close to the chiral center having π electrons or non-bonding electron pairs (in the appropriate position) can cause the same electrostatic effects (Table 4.1, entries 8-11). Finally, induction will decrease when the methyl group at the chiral center is replaced by a larger alkyl group due to decreasing steric difference between both sides of the molecule (Table 4.1, entries 5-7).

4.4 Attempted synthesis towards chiral (+)-daunomycinone.

In order to apply chiral dienes in the synthesis of (+)-daunomycinone, preliminary studies were carried out to synthesize the optically active Diels-Alder adducts (+)- and (-)-**9g** . They were prepared from dienes (+)- and (-)-**4g** having either (+)- or (-)-phenylethoxy groups (Scheme 4.4) and they were converted into the (+) and (-) form of **22** in the same way as described above for diene **4** (R = *t*-Bu, Chapter 3, Scheme 3.3).

It appeared that, after deprotection of **22**, chirality was almost lost. This is most probably due to the strong acidic conditions necessary for removal of the protecting group. Indeed, when chiral (+)-daunomycinone was treated with trifluoroacetic acid for 15 minutes at room temperature, chirality was diminished by about 50%. It has been reported that anthracyclines undergo epimerization at the 7 position of the A ring in the presence of trifluoroacetic acid[17]. This result suggests that for the synthesis of chiral compound **23**, it will be better to apply the chiral diene **4n** with R_4= Me and R_5= p-$MeOC_6H_4$. In this case, higher induction can be expected, and the chiral group can also be removed under milder acidic conditions.

Scheme 4.4



2 **4g** **9g** **21#** **22#** **23#** **24**

4.5 Conclusions.

An essential step in the total synthesis of daunomycin and its derivatives is the introduction of 2 chiral centers at the 7 and 9 positions of the daunomycinone. After coupling with the chiral sugar daunosamine, in most cases it is possible to separate the 7S,9S and 7R,9R diastereomers (Chapters 5 and 6), but the biologically active isomer (7S,9S) can only be isolated in no more than 50% yield. Therefore, introduction of chirality at an early stage of the total synthesis is preferred for successful synthesis of new derivatives. In this chapter, we have shown that it is possible to obtain a reasonable to high diastereomeric excess in the Diels-Alder reactions of quinones, such as naphthazarin, juglone, naphthaquinone and benzoquinone using chiral 1-alkoxy-3-trimethylsilyloxybuta-1,3-dienes. The highest diastereomeric excess was obtained using 1-[1-(*p*-methoxyphenyl)-ethoxy]-3-trimethylsilyloxybuta-1,3-diene in the reaction with benzoquinone. Only one isomer was detected using ^{1}H-NMR spectroscopy. We tried to use both (+)- and (-)-1-[1-phenyl-ethoxy]-3-trimethylsilyloxybuta-1,3-dienes in the Diels-Alder reaction with napthazarin in order to synthesize chiral anthracyclines. After introduction of the 1-trimethylsilylethynyl group (**9g** to **21**, Scheme 4.4), the desired 1S,3S or 1R,3R isomers were obtained. However, we have not yet been able to obtain the desired optically active daunomycinone (**24**), as shown in Scheme 4.4, due to epimerization upon removal of the chiral alkoxy group under (the necessary strong) acidic conditions.

4.6 Experimental section.

General remarks:
^{1}H-NMR-spectra were measured either on a Bruker WH-90 spectrometer or an Bruker AM-400 spectrometer using Me_4Si as internal standard. $CDCl_3$ was used as solvent unless otherwise stated. Melting points were taken using a Reichert Thermpan microscope and are uncorrected. Mass spectra were taken using a double focusing VG 7070 E mass spectrometer. For column chromatography, either Merck Silicagel 60 or Merck silicagel Art. 9385 (flash chromatography) were used. Dry tetrahydrofuran was distilled from sodium benzophenone ketyl.

Synthesis of 1-alkoxy-1-buteen-3-ones **13** (general procedure).

The alcohols (200 mmol) were dissolved in 250 ml of dry toluene and 300-400 mmol of 1-methoxy-1-buteen-3-one (**12**) were added. A catalytic amount of *p*-toluenesulfonic acid or sodium methoxide was added, depending on the type of alcohol used. The reaction mixture was refluxed for 1-3 hours (acid-catalysed) or 10-17 hours (base-catalysed). The formed methanol was captured with 4Å molecular sieves in Dean-Stark equipment. After the reaction was completed, Et_3N was added (acid-catalysed reaction) and the mixture was evaporated *in vacuo*. The residue was distilled *in vacuo* and yielded the 1-alkoxy-buten-3-ones **13** **c-r**. Yields, boiling points and ^{1}H-NMR shifts are given below for entries 1 - 16 (Scheme 4.2 and Table 4.1/4.2):

entry 1: **13c**, Yield; 86 %; b.p.: 59-61°C at 3 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.3 ppm, 1H, d, H_1; δ=5.5 ppm, 1H, d, H_2; δ=2.5 ppm, 1H, s, CH; δ=2.1 ppm, 3H, s, $COCH_3$; δ=1.1 ppm, 3H, d, CH_3; δ=0.9 ppm, 2H, m, CH_2; δ=0.8 ppm, 3H, t, CH_3.

entry 2: **13d**, Yield 78%; b.p.: 80-82°C at 3 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.3 ppm, 1H, d, H_1; δ=5.5 ppm, 1H, d, H_2; δ=3.2 ppm, 1H, m, CH; δ=2.1 ppm, 3H, s, $COCH_3$; δ=1.6 ppm, 1H, m, CH; δ=1.1 ppm, 3H, d, CH_3; δ=0.8 ppm, 6H, d, CH_3.

entry 3: **13e**, Yield 91%; b.p.: 79-82°C at 1 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.3 ppm, 1H, d, H_1; δ=5.4 ppm, 1H, d, H_2; δ=2.2 ppm, 1H, m, CH; δ=2.1 ppm, 3H, s, $COCH_3$; δ=1.1 ppm,

3H, d, CH_3; δ=0.7 ppm, 9H, d, $C(CH_3)_3$.
entry 4: 13f, Yield 62%; b.p.: 109-110°C at 0.4 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.5 ppm, 1H, d, J=11 Hz, H_1; δ=5.6 ppm, 1H, d, J=11 Hz, H_2; δ=3.8 ppm, 1H, m, CH; δ=2.27 ppm, 3H, s, $COCH_3$; δ=1.25 ppm, 3H, d, J=6 Hz, CH_3; δ=1.0-2.5 ppm, 11H, m, C_6H_{11}.
entry 5: 13g, Yield 84%; b.p.: 95-99°C at 0.2 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.7 ppm, 1H, d, J=12.4 Hz, H_1; δ=7.2 ppm, 5H, s, C_6H_5; δ=5.5 ppm, 1H, d, J=12.4 Hz, H_2; δ=4.7 ppm, 1H, q, J=6.4 Hz, CH; δ=2.1 ppm, 3H, s, $COCH_3$; δ=1.6 ppm, 3H, d, J=6.4 Hz, CH_3.
entry 6: 13h, Yield 80%; b.p.: 108-111°C at 0.3 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.3 ppm, 1H, d, H_1; δ=7.1 ppm, 5H, s, C_6H_5; δ=5.5 ppm, 1H, d, H_2; δ=4.7 ppm, 1H, t, CH; δ=2.0 ppm, 3H, s, $COCH_3$; δ=1.9 ppm, 2H, m, CH_2; δ=0.9 ppm, 3H, d, CH_3.
entry 7: 13i, Yield 75%; b.p.: 105-107°C at 0.3 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.2 ppm, 1H, d, H_1; δ=7.1 ppm, 5H, s, C_6H_5; δ=5.5 ppm, 1H, d, H_2; δ=4.3 ppm, 2H, m, 2 x CH; δ=2.0 ppm, 3H, s, $COCH_3$; δ=0.9 ppm, 6H, d, CH_3.
entry 8: 13j, Yield 94%; b.p.: 80-82°C at 2 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.4 ppm, 1H, d, H_1; δ=5.6 ppm, 1H, d, H_2; δ=4.1 ppm, 1H, m, CH; δ=3.4 ppm, 2H, d, OCH_2; δ=3.3 ppm, 3H, s, OCH_3; δ=2.2 ppm, 3H, s, $COCH_3$; δ=1.3 ppm, 3H, d, CH_3.
entry 9: 13k, Yield 78%; b.p.: 70-78°C at 3 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.4 ppm, 1H, d, H_1; δ=5.6 ppm, 1H, d, H_2; δ=5.5-5.3 ppm, 3H, m, $HC=CH_2$; δ=3.3 ppm, 3H, s, $COCH_3$; δ=1.3 ppm, 3H, d, CH_3.
entry 10: 13l, Yield 52%; b.p.: 86-93°C at 0.25 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.5 ppm, 1H, d, J=12.5 Hz, H_1; δ=5.6 ppm, 1H, d, J=12.5 Hz, H_2; δ=4.3 ppm, 1H, m, HCO; δ=3.6 ppm, 2H, t, J=6 Hz, CH_2; δ=3.37 ppm, 3H, s, OCH_3; δ=2.2 ppm, 3H, s, $COCH_3$; δ=1.80 ppm, 2H, m, CH_2; δ=1.33 ppm, 3H, d, CH_3.
entry 11: 13m, Yield 88%; b.p.: 99-101°C at 0.2 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.4 ppm, 1H, d, H_1; δ=7.1 ppm, 5H, s, ArH; δ=5.5 ppm, 1H, d, H_2; δ=3.9 ppm, 2H, d, CH_2; δ=3.1 ppm, 1H, m, CH; δ=3.1 ppm, 3H, s, $COCH_3$; δ=1.3 ppm, 3H, d, CH_3.
entry 12: 13n, Yield 84%; b.p.: 139-142°C at 0.35 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.52 ppm, 1H, d, J=13 Hz, H_1; δ=7.27 ppm, 2H, dd, J=8.5 Hz, ArH; δ=6.78 ppm, 2H, dd, J=8.5 Hz, ArH; δ=5.63 ppm, 1H, d, J=13 Hz, H_2; δ=5.05 ppm, 1H, q, J=6.3 Hz, CH; δ=3.78 ppm, 3H, s, OCH_3; δ=2.07 ppm, 3H, s, $COCH_3$; δ=1.57 ppm, 3H, d, J=6.3 Hz, CH_3.
entry 13: 13o, Yield 80%; b.p.: 122-128°C at 0.55 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.48 ppm, 1H, d, J=12.4 Hz, H_1; δ=7.17 ppm, 4H, s, ArH; δ=5.62 ppm, 1H, d, J=12.4 Hz, H_2; δ=4.70 ppm, 1H, q, J=6.3 Hz, CH; δ=2.33 ppm, 3H, s, CH_3; δ=2.07 ppm, 3H, s, $COCH_3$; δ=1.57 ppm, 3H, d, J=6.3 Hz, CH_3.
entry 14: 13p, Yield 38%; b.p.: 110-120°C at 0.2 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.63 ppm, 1H, d, J=12.4 Hz, H_1; δ=7.27 ppm, 4H, m, ArH; δ=5.57 ppm, 1H, d, J=12.4 Hz, H_2; δ=5.03 ppm, 1H, q, J=6.3 Hz, CH; δ=2.08 ppm, 3H, s, $COCH_3$; δ=1.52 ppm, 3H, d, J=6.3 Hz, CH_3.
entry 15: 13q, Yield 79%; b.p.: 123-127°C at 0.2 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.28-7.50 ppm, 5H, m, H_1 + ArH; δ=5.60 ppm, 1H, d, J=13 Hz, H_2; δ=5.15 ppm, 1H, q, J=6.3 Hz, CH; δ=2.12 ppm, 3H, s, $COCH_3$; δ=1.63 ppm, 3H, d, J=6.3 Hz, CH_3.
entry 16: 13r, Yield 97%; b.p.: 131°C at 0.2 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.50 ppm, 1H, d, J=13 Hz, H_1; δ=7.49 ppm, 4H, dd, J=8 Hz, ArH; δ=5.58 ppm, 1H, d, J=13 Hz, H_2; δ=4.88 ppm, 1H, t, J=7 Hz, CH; δ=2.08 ppm, 3H, s, $COCH_3$; δ=1.82, 2H, m, CH_2; δ=0.93 ppm, 3H, t, J=7 Hz, CH_3.

Synthesis of 1-alkoxy-3-trimethylsilyloxy-1,3-butadienes **4** (general procedure).

The buten-3-ones **13** (67.4 mmol) were dissolved in 60 ml of dry acetonitrile. Triethylamine (21.2 g, 209 mmol) and trimethylsilylchloride (10.5 g, 96.6 mmol) were added and the reaction mixture was stirred at room temperature for one hour. The solvent was evaporated *in vacuo* and the residue was then dissolved in n-pentane. After filtration, the precipitate was washed with n-pentane and the combined organic layers were then evaporated *in vacuo*. The light brown residue was distilled under reduced pressure to give the 1-alkoxy-3-trimethylsilyloxybuta--1,3-dienes (**4**). Yields, boiling points and ^{1}H-NMR shifts are given for entries 1-16 (Scheme 4.2 and Table 4.1/4.2) are given below:
entry 1: 4c, Yield; 83%; b.p.: 59-60°C at 1 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=6.6 ppm, 1H, d, H_1; δ=5.4 ppm, 1H, d, H_2; δ=3.9 ppm, 2H, s, H_4; δ=2.5 ppm, 1H, s, CH; δ=1.1 ppm, 3H, d, CH_3; δ=0.9 ppm, 2H, m, CH_2; δ=0.8 ppm, 3H, t, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.

entry 2: 4d, Yield 88%; b.p.: 65-70°C at 1.5 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=6.5 ppm, 1H, d, H_1; δ=5.4 ppm, 1H, d, H_2; δ=3.9 ppm, 2H, s, H_4; δ=2.1 ppm, 1H, m, CH; δ=1.6 ppm, 1H, m, CH; δ=1.1 ppm, 3H, d, CH_3; δ=0.9 ppm, 6H, d, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 3: 4e, Yield 74%; b.p.: 80-82°C at 1 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=6.5 ppm, 1H, d, H_1; δ=5.3 ppm, 1H, d, H_2; δ=4.0 ppm, 2H, s, H_4; δ=2.2 ppm, 1H, m, CH; δ=1.1 ppm, 3H, s, $COCH_3$; δ=0.7 ppm, 9H, d, $C(CH_3)_3$; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 4: 4f, Yield 52%; b.p.: 97-101°C at 0.6 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.47 ppm, 1H, d, J=13 Hz, H_1; δ=5.57 ppm, 1H, d, J=13 Hz, H_2; δ=4.0 ppm, 2H, s, H_4; δ=2.1 ppm, 1H, m, CH; δ=1.22 ppm, 3H, d, J=6 Hz, CH_3; δ=0.9-2.0 ppm, 11H, d, C_6H_{11}; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 5: 4g, Yield 81%; b.p.: 115-120°C at 0.1 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.17 ppm, 5H, s, ArH; δ=6.53 ppm, 1H, d, J=12.4 Hz, H_1; δ=5.39 ppm, 1H, d, J=12.4 Hz, H_2; δ=4.80 ppm, 1H, q, J=6.3 Hz, CH; δ=3.96 ppm, 2H, s, H_4; δ=1.60 ppm, 3H, d, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 6: 4h, Yield 80%; b.p.: 119-121°C at 0.1 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.0 ppm, 5H, s, ArH; δ=6.4 ppm, 1H, d, H_1; δ=5.2 ppm, 1H, d, H_2; δ=4.5 ppm, 1H, t, CH; δ=3.8 ppm, 2H, s, H_4; δ=1.8 ppm, 2H, m, CH_2; δ=0.8 ppm, 3H, d, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 7: 4i, Yield 72%; b.p.: 110-115°C at 0.1 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.0 ppm, 5H, s, ArH; δ=6.4 ppm, 1H, d, H_1; δ=5.2 ppm, 1H, d, H_2; δ=4.5 ppm, 1H, t, CH; δ=3.8 ppm, 2H, s, H_4; δ=1.9 ppm, 1H, m, CH; δ=1.0 ppm, 6H, d, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 8: 4j, Yield 94%; b.p.: 81°C at 1 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=6.6 ppm, 1H, d, H_1; δ=5.3 ppm, 1H, d, H_2; δ=4.0 ppm, 2H, bs, H_4; δ=3.4 ppm, 2H, d, OCH_2; δ=3.3 ppm, 3H, s, OCH_3; δ=2.2 ppm, 1H, m, CH; δ=1.3 ppm, 3H, d, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 9: 4k, Yield 87%; b.p.: 55-56°C at 0.3 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=6.5 ppm, 1H, d, H_1; δ=5.2 ppm, 1H, d, H_2; δ=5.0-4.5 ppm, 3H, m, $HC=CH_2$; δ=4.0 ppm, 2H, bs, H_4; δ=1.3 ppm, 3H, d, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 10: 4l, Yield 44%; b.p.: 92°C at 0.2 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=6.6 ppm, 1H, d, J=12.5 Hz, H_1; δ=5.33 ppm, 1H, d, J=12.5 Hz, H_2; δ=4.10-3.73 ppm, 2H, s, H_4 and 1H, q, J=6 Hz, HCO; δ=3.42 ppm, 2H, t, J=6 Hz, CH_2; δ=3.27 ppm, 3H, s, OCH_3; δ=1.73 ppm, 2H, m, CH_2; δ=1.18 ppm, 3H, d, CH_3; δ=0.22 ppm, 9H, s, $OSi(CH_3)_3$.
entry 11: 4m, Yield 76%; b.p.: 111°C at 0.1 mm Hg; ^{1}H-NMR (60 MHz, $CDCl_3$): δ=7.1 ppm, 5H, s, ArH; δ=6.6 ppm, 1H, d, H_1; δ=5.2 ppm, 1H, d, H_2; δ=4.0 ppm, 2H, s, H_4; δ=3.9 ppm, 2H, d, CH_2; δ=2.1 ppm, 1H, s, CH; δ=1.3 ppm, 3H, d, CH_3; δ=0.2 ppm, 9H, s, $OSi(CH_3)_3$.
entry 12: 4n, Yield 71%; b.p.: 145°C at 0.35 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.23 ppm, 2H, d, J=8.4 Hz, ArH; δ=6.84 ppm, 2H, d, J=8.4 Hz, ArH; δ=6.67 ppm, 1H, d, J=12.4 Hz, H_1; δ=5.43 ppm, 1H, d, J=12.4 Hz, H_2; δ=4.82 ppm, 1H, q, J=6.3 Hz, CH; δ=4.03 ppm, 2H, s, H_4, δ=3.75 ppm, 3H, s, OCH_3; δ=1.50 ppm, 3H, d, J=6.3 Hz, CH_3; δ=0.17 ppm, 9H, s, $OSi(CH_3)_3$.
entry 13: 4o, Yield 76%; b.p.: 120-130°C at 0.1 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.13 ppm, 4H, s, ArH; δ=6.61 ppm, 1H, d, J=12.4 Hz, H_1; δ=5.39 ppm, 1H, d, J=12.4 Hz, H_2; δ=4.80 ppm, 1H, q, J=6.3 Hz, CH; δ=4.00 ppm, 2H, s, H_4; δ=2.32 ppm, 3H, s, CH_3; δ=1.53 ppm, 3H, d, J=6.3 Hz, CH_3; δ=0.17 ppm, 9H, s, $OSi(CH_3)_3$.
entry 14: 4p, Yield 34%; b.p.: 127°C at 0.5 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.24 ppm, 4H, m, ArH; δ=6.60 ppm, 1H, d, J=12 Hz, H_1; δ=5.42 ppm, 1H, d, J=12 Hz, H_2; δ=4.83 ppm, 1H, q, J=6.3 Hz, CH; δ=4.05 ppm, 2H, s, H_4; δ=1.53 ppm, 3H, d, J=6.3 Hz, CH_3; δ=0.17 ppm, 9H, s, $OSi(CH_3)_3$.
entry 15: 4q, Yield 80%; b.p.: 123-127°C at 0.5 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.53 ppm, 4H, dd, J=4 and J=8 Hz, ArH; δ=6.67 ppm, 1H, s, H_1; δ=5.46 ppm, 1H, d, J=13 Hz, H_2; δ=4.82 ppm, 1H, q, J=6.3 Hz, CH; δ=3.93 ppm, 2H, s, H_4; δ=1.39 ppm, 3H, d, J=6.3 Hz, CH_3; δ=0.17 ppm, 9H, s, $OSi(CH_3)_3$.
entry 16: 4r, Yield 75%; b.p.: 125°C at 0.65 mm Hg; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=7.57 ppm, 4H, d, J=8 Hz, ArH; δ=6.67 ppm, 1H, d, J=13 Hz, H_1; δ=5.43 ppm, 1H, d, J=13 Hz, H_2; δ=4.70 ppm, 1H, t, J=7 Hz, CH; δ=4.08 ppm, 2H, s, H_4; δ=1.83, 2H, m, CH_2; δ=0.93 ppm, 3H, t, J=7 Hz, CH_3; δ=0.17 ppm, 9H, s, $OSi(CH_3)_3$.

Reaction of dienes **4** *with naphthazarin* **1**, *juglone* **2**, *naphthoquinone* **12** *and benzoquinone* **14** (General procedure).

Under an argon atmosphere diene **4** (6.4 mmol; 1.5 equiv.) was added to a solution of dienophiles (**1**, **2**, **14** and **15**, 4.2 mmol) in 40 ml of dry THF. The reaction mixture was stirred at room temperature for 16-20 h. Progress of the reaction was followed by TLC (ethyl

acetate/n-hexane, 3 : 5). To convert the primary cycloadduct into the more stable ketones (**9**, **8**, **16** and **17**), the cycloadduct was hydrolysed at 0°C by the addition of 2 ml of 1N hydrogen chloride. After stirring at 0°C for 15 minutes the reaction was quenched by the addition of 50 ml of water. The water layer was extracted twice with 50 ml of chloroform and the combined organic layers were then dried over anhydrous sodium sulfate. After evaporation *in vacuo*, the residue was dissolved in a small amount of THF (usually 5 ml) and the solution was then added to a vigorously stirred solution of n-hexane (75 ml, ratio THF : n-hexane ~ 1 : 15). The precipitated cycloadducts (**9**, **8**, **15** and **16**) were filtered off and washed with n-hexane. Isolated yields of the cycloadducts were 50% - 60%. Melting points, ^{1}H-NMR shifts and mass spectra are given for entries 1-16 (Scheme 4.1 and Table 4.1/4.2) below.

entry 1: **8c**, Yield 72%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.4 ppm, 1H, s, ArOH; δ=8.0 ppm, 2H, m, ArH; δ=7.2 ppm, 1H, m, ArH; δ=4.6 ppm, 1H, m, CH; δ=3.9 ppm, 3H, m; δ=3.6 ppm, 1 H, m; δ=2.7-3.1 ppm, 3H, m; δ=0.3-1.4 ppm, 8H, m, contains 1.45H, d, J=6.3 Hz, CH_3 and 1.55H, d, J=6.3 Hz, CH_3.

entry 2: **8d**, Yield 74%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.5 ppm, 1H, s, ArOH; δ=7.8 ppm, 2H, m, ArH; δ=7.2 ppm, 1H, m, ArH; δ=4.5 ppm, 1H, m, CH; δ=3.7 ppm, 3H, m; δ=3.0-3.2 ppm, 1H, m; δ=2.3-2.8 ppm, 3H, m; δ=1.5-1.0 ppm, 1H, m; δ=0.9 ppm, 1.41H, d, J=6.3 Hz, CH_3; δ=0.2-0.6 ppm, contains: 1.59 H, d, J=6.3 Hz, CH_3 and 3H, m, CH_3.

entry 3: **8e**, Yield 78%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.5 ppm, 1H, s, ArOH; δ=7.2-7.8 ppm, 3H, m, ArH; δ=4.5 ppm, 1H, m CH; δ=3.6 ppm, 3H, m; δ=2.4-3.1 ppm, 4H, m; δ=0.9 ppm, 1.27H, d, J=6.3 Hz, CH_3; δ=0.6 ppm, 1.73H, d, J=6.3 Hz, CH_3; δ=0.5 ppm, 9H, ds, $C(CH_3)_3$.

entry 4: **8f**, Yield 45%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.5 ppm, 1H, s, ArOH; δ=7.2-7.8 ppm, 3H, m, ArH; δ=4.5 ppm, 1H, m, CH; δ=3.7 ppm, 3H, m; δ=3.0-3.2 ppm, 1H, m; δ=2.3-2.8 ppm, 3H, m; δ = 1.0-2.2 ppm, 11H, C_6H_{11}; δ=0.9 ppm, 1.37H, d, J=6.3 Hz, CH_3; δ=0.5 ppm, 1.63H, d, J=6.3 Hz, CH_3.

entry 5: **8g**, Yield 79%; m.p. 140°C (dec.); m/e: 364 (M^+), 244, 184, 105; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.22 ppm, 0.83H, s, ArOH; δ=11.97 ppm, 0.17H, s, ArOH; δ=7.61 ppm, 2H, m, ArH; δ=7.21 ppm, 6H, m, ArH; δ=4.31 ppm, 1H, m, HCO; δ=3.90 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.50 ppm, 3H, m; δ=2.43 ppm, 1H, dd; δ=2.32 ppm, 2H, dd; δ=1.01 ppm, 0.51H, d, J=6.3 Hz, CH_3; δ=0.66 ppm, 2.49H, d, J=6.3 Hz, CH_3.

entry 6: **8h**, Yield 76%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.5 ppm, 0.80H, s, ArOH; δ=12.2 ppm, 0.20H, s, ArOH; δ=7.9 ppm, 2H, m, ArH; δ=7.4 ppm, 5H, m, ArH; δ=7.1 ppm, 1H, m, ArH; δ=4.5 ppm, 1H, m, HCO; δ=3.4-3.9 ppm, 4H, m; δ=2.3-2.6 ppm, 3H, m; δ=1.0-1.4 ppm, 2H, m, CH_2; δ=0.6 ppm, 0.6H, t, J=7.2 Hz, CH_3; δ=0.4 ppm, 2.4H, t, J=7.2 Hz, CH_3.

entry 7: **8i**, Yield 86%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.5 ppm, 0.75H, s, ArOH; δ=12.3 ppm, 0.25H, s, ArOH; δ=7.9 ppm, 1.5H, m, ArH; δ=7.4 ppm, 5H, m, ArH; δ=7.1 ppm, 1.5H, m, ArH; δ=4.6 ppm, 1H, m; δ=3.5-3.9 ppm, 4H, m; δ=2.2-2.7 ppm, 3H, m; δ=1.4 ppm, 1H, m; δ=0.2-0.5 ppm, 9H, m, contains: 6H, d, J=6.2 Hz, 2 x CH_3;

entry 8: **8j**, Yield 77%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.7 ppm, 1H, s, ArOH; δ=8.0 ppm, 2H, m, ArH; δ=7.7 ppm, 1H, m, ArH; δ=4.9 ppm, 0.35H, m HCO; δ=4.8 ppm, 0.65H, m, HCO; δ=3.8 ppm, 3H, s, OCH_3; δ=3.6 ppm, 2H, bd, CH_2; δ=2.7-4.3 ppm, 6H, m; δ=1.1 ppm, 1.05H, d, J= 6.3 Hz, CH_3; δ=0.7 ppm, 1.95H, d, J= 6.3 Hz, CH_3.

entry 9: **8k**, Yield 76%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.51 ppm, 0.73H, s, ArOH; δ=12.40 ppm, 0.27H, s, ArOH; δ=7.3-7.9 ppm, 3H, m, ArH; δ=5.4-5.9 ppm, 1H, m, C=CH; δ=4.8-5.2 ppm, 2H, m, $C=CH_2$; δ=4.4 ppm, 1H, m, HCO; δ=3.5-3.8 ppm, 3H, m; δ=2.3-2.7 ppm, 3H, m; δ=0.8 ppm, 0.81H, d, J=6.3 Hz, CH_3; δ=0.5 ppm, 2,19H, d, J=6.3 Hz, CH_3.

entry 10: **8l**, Yield 63%; m.p. 95°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.44 ppm, 0.5H, s, ArOH; δ=12.40 ppm, 0.5H, s, ArOH; δ=7.92 ppm, 2H, m, ArH; δ=7.53 ppm, 1H, m, ArH; δ=4.33 ppm, 1H, m, HCO; δ=3.75 ppm, 1H, m; δ=3.64 ppm, 3H, s, OCH_3; δ=3.33-3.50 ppm, 2H, t, J=5 Hz, OCH_2; δ=2.58-3.28 ppm, 3H, m; δ=1.29-1.65 ppm, 2H, d, J=6.3 Hz and t, J=5 Hz, CH_2; δ=1.19 ppm, 1.50H, d, J=6.3 Hz, CH_3; δ=0.89 ppm, 1.50H, d, J=6.3 Hz, CH_3.

entry 11: **8m**, Yield 88%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.4 ppm, 0.53H, s, ArOH; δ=12.2 ppm, 0.47H, s, ArOH; δ=7.9 ppm, 2H, m, ArH; δ=7.3 ppm, 5H, m, ArH; δ=7.0 ppm, 1H, m, ArH; δ=4.4 ppm, 1H, m, 2HCO; δ=2.8-3.8 ppm, 4H, m; δ=2.5 ppm, 2H, d, J=2.5 Hz, CH_2; δ=2.3-2.6 ppm, 3H, m; δ=0.9 ppm, 1.59H, d, J=6.8 Hz, CH_3; δ=0.7 ppm, 1.41H, d, J=6.3 Hz, CH_3.

entry 12: **8n**, Yield 40%; m.p. 141°C; m/e: 394 (M^+), 243, 226, 197, 151, 135; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.22 ppm, 0.84H, s, ArOH; δ=12.00 ppm, 0.16H, s, ArOH; δ=7.63 ppm, 1H, ABC, ArH; δ=7.28 ppm, 1H, AB, ArH; δ=6.88-7.03 ppm, 4H, m, ArH; δ=4.29 ppm, 1H, m,

HCO; δ=3.86 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.76 ppm, 3H, s, OCH_3; δ=3.38-3.67 ppm, 3H, m; δ=2.45 ppm, 1H, dd, J=6.3 Hz; δ=2.33 ppm, 2H, d, J=2.5 Hz; δ=0.99 ppm, 0.48H, d, J=6.3 Hz, CH_3; δ=0.66 ppm, 2.52H, d, J=6.3 Hz, CH_3.

entry 13: 8o, Yield 55%; m.p. 140°C (dec.); m/e: 378 (M^+), 243, 212, 197, 119; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.25 ppm, 0.84H, s, ArOH; δ=12.03 ppm, 0.16H, s, ArOH; δ=7.63 ppm, 2H, dd, AB, ArH; δ=7.28 ppm, 2H, dd, AB, J=5 Hz, ArH; δ=6.81-7.14 ppm, 3H, dd, J=7.5Hz, ArH; δ=4.32 ppm, 1H, m, HCO; δ=3.87 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.40-3.64 ppm, 3H m; δ=2.42 ppm, 1H, dd, J=7.5 Hz; δ=2.19-2.34 ppm, 5H, m; δ=0.95 ppm, 0.49H, d, J=6.3 Hz, CH_3; δ=0.65 ppm, 2.51H, d, J=6.3 Hz, CH_3.

entry 14: 8p, Yield 44%; m.p. 112-115°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.20 ppm, 0.81H, s, ArOH; δ=11.98 ppm, 0.19H, s, ArOH; δ=7.57-7.70 ppm, 3H, m, ArH; δ=7.21-7.27 ppm, 4H, m, ArH; δ=4.34 ppm, 1H, m, HCO; δ=3.95 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.49-3.63 ppm, 3H, m; δ=2.38 ppm, 3H, m; δ=0.98 ppm, 0.57H, d, J=6.3 Hz, CH_3; δ=0.67 ppm, 2.43H, d, J=6.3 Hz, CH_3.

entry 15: 8q, Yield 53%; m.p. 114°C; m/e: 432 (M^+), 259, 244, 215, 173; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.21 ppm, 0.73H, s, ArOH; δ=11.98 ppm, 0.27H, s, ArOH; δ=7.67 ppm, 2H, m, ArH; δ=7.53 ppm, 2H, dd, J=8 Hz, ArH; δ=7.28 ppm, 1H, m, ArH; δ=7.08 ppm, 2H, dd, J=8 Hz, ArH; δ=4.39 ppm, 1H, m, HCO; δ=4.11 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.42-3.75 ppm, 3H, m; δ=2.57 ppm, 1H, dd, J=3 Hz; δ=2.42 ppm, 2H, dd, J=3 Hz and J=7 Hz; δ=1.04 ppm, 0.81H, d, J=6.3 Hz, CH_3; δ=0.72 ppm, 2.19H, d, J=6.3 Hz, CH_3.

entry 16: 8r, Yield 78%; m.p. 115-116°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.22 ppm, 0.71H, s, ArOH; δ=11.99 ppm, 0.29H, s, ArOH; δ=7.65 ppm, 1H, m, ArH; δ=7.54 ppm, 2H, d, J=8 Hz, ArH; δ=7.26 ppm, 2H, m, ArH; δ=7.06 ppm, 2H, d, J=8 Hz, ArH; δ=4.44 ppm, 1H, m, HCO; δ=3.94 ppm, 1H, t, J= 6.3 Hz, HCO; δ=3.39-3.71 ppm, 3H, m; δ=2.31-2.60 ppm, 3H, m; δ=1.17 ppm, 2H, m, CH_2; δ=0.62 ppm, 0.87H, t, J=7 Hz, CH_3; δ=0.19 ppm, 2.13H, t, J=7 Hz, CH_3.

entry 1: 9c, Yield; 71%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.1 ppm, 1H, s, ArOH; δ=11.8 ppm, 1H, s, ArOH; δ=7.3 ppm, 2H, bs, ArH; δ=4.4 ppm, 1H, m, HCO; δ=3.4 ppm, 3H, m; δ=3.2 ppm, 3H, m; δ=0.3-1.4 ppm, 8H, m, contains 1.4 H, d, J=6.3 Hz, CH_3 and 1.6 H, d, J=6.3 Hz, CH_3.

entry 2: 9d, Yield 75%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.1 ppm, 1H, s, ArOH; δ=11.8 ppm, 1H, s, ArOH; δ=7.2 ppm, 2H, bs, ArH; δ=4.4 ppm, 1H, m, HCO; δ=3.4-3.8 ppm, 3H, m; δ=3.0-3.3 ppm, 1H, m; δ=2.3-3.0 ppm, 3H, m; δ=1.5 ppm, 1H, m; δ=0.8 ppm, 1.28 H, d, J=6.2 Hz, CH_3; δ=0.3-0.7 ppm, contains 1.72 H, d, J=6.2 Hz, CH_3, 2.56 H, d, 2 CH_3 and 3.44 H, d, 2 CH_3.

entry 3: 9e, Yield 69%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.03 ppm, 0.65H, s, ArOH; δ=12.01 ppm, 0.35H, s, ArOH; δ=11.52 ppm, 0.35H, s, ArOH; δ=11.51 ppm, 0.65H, s, ArOH; δ=7.2 ppm, 2H, bs, ArH; δ=4.4 ppm, 1H, m, HCO; δ=3.5 ppm, 3H, m; δ=2.3-3.0 ppm, 4H, m; δ=1.8 ppm, 1.05H, d, J=6.3 Hz, CH_3; δ=1.5 ppm, 1H, m; δ=0.3-0.5 ppm, contains 1.95 H, d, J=6.3 Hz, CH_3, 5.85 H, s, $O(CH_3)_3$ and 3.15 H, s, $O(CH_3)_3$.

entry 4: 9f, Yield 63%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.1 ppm, 1H, s, ArOH; δ=11.8 ppm, 1H, s, ArOH; δ=7.2 ppm, 2H, bs, ArH; δ=4.4 ppm, 1H, m, HCO; δ=3.4-3.8 ppm, 3H, m; δ=3.0-3.3 ppm, 1H, m; δ=2.3-3.0 ppm, 3H, m; δ = 1.0-2.2 ppm, 11H, C_6H_{11}; δ=0.8 ppm, 1.37H, d, J=6.2 Hz, CH_3; δ=0.4 ppm, 1.63H, d, J=6.2 Hz, CH_3.

entry 5: 9g, Yield 62%; m.p. 138-139.5°C; m/e: 380 (M^+), 276, 258, 105; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.11 ppm, 1H, s, ArOH; δ=11.88 ppm, 0.15H, s, ArOH; δ=11.58 ppm, 0.85H, s, ArOH; δ=7.25 ppm, 5H, s, ArOH; δ=7.07 ppm, 1H, s, ArH; δ=7.02 ppm, 1H, s, ArH; δ=4.33 ppm, 1H, m, HCO; δ=3.97 ppm, 1H, q, J=6.3 Hz, CH; δ=3.39-3.69 ppm, 3H, m; δ=2.52 ppm, 1H, dd, J=8 Hz; δ=2.42 ppm, 2H, dd, J=8 Hz and J=4 Hz; δ=1.08 ppm, 0.45H, d, J=6.3 Hz, CH_3; δ=0.81 ppm, 2.55H, d, J=6.3 Hz, CH_3.

entry 6: 9h, Yield 67%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.3 ppm, 0.75H, s, ArOH; δ=12.0 ppm, 0.25H, s, ArOH; δ=11.82 ppm, 0.25H, s, ArOH; δ=11.78 ppm, 0.75H, s, ArOH; δ=7.30 ppm, 5H, s, ArOH; δ=7.1 ppm, 2H, s, ArH; δ=4.4 ppm, 1H, m, HCO; δ=3.4-3.9 ppm, 4H, m; δ=2.3-2.6 ppm, 3H, m; δ=1.0-1.4 ppm, 2H, m, CH_2; δ=0.6 ppm, 0.75H, t, J=7.4 Hz, CH_3; δ=0.4 ppm, 2.25H, t, J=7.4 Hz, CH_3.

entry 7: 9i, Yield 48%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.4 ppm, 0.65H, s, ArOH; δ=12.1 ppm, 0.35H, s, ArOH; δ=11.8 ppm, 1H, s, ArOH; δ=7.4 ppm, 5H, s, ArOH; δ=7.1 ppm, 1H, m, ArH; δ=6.7 ppm, 1H, m, ArH; δ=4.5 ppm, 1H, m, HCO; δ=3.4-3.9 ppm, 4H, m; δ=2.2-2.7 ppm, 3H, m; δ=1.2-1.6 ppm, 1H, m; δ=0.6 ppm, 1.05H, d, J=6.2 Hz, $2CH_3$; δ=0.2-0.5 ppm, m, CH_3.

entry 8: 9j, Yield 80%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.31 ppm, 0.33H, s, ArOH; δ=12.29 ppm, 0.67H, s, ArOH; δ=11.9 ppm, 1H, s, ArOH; δ=7.4 ppm, 2H, bs, ArH; δ=4.5 ppm, 1H, m,

HCO; δ=2.3-3.8 ppm, 6H, m; δ=3.2 ppm, 3H, s, OCH_3; δ=2.9 ppm, 2H, d, J=5 Hz, OCH_2; δ=1.7 ppm, 1H, m; δ=0.8 ppm, 1H, d, J=6.3 Hz, CH_3; δ=0.5 ppm, 2H, d, J=6.3 Hz, CH_3.

entry 9: 9k, Yield 62%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.30 ppm, 0.75H, s, ArOH; δ=12.29 ppm, 0.25H, s, ArOH; δ=12.0 ppm, 1H, s, ArOH; δ=7.4 ppm, 2H, m, ArH; δ=5.4 ppm, 1H, m, HC=C; δ=4.9-5.1 ppm, 2H, m, H_2C=C; δ=4.4 ppm, 1H, m, HCO; δ=3.3-3.9 ppm, 3H, m; δ=2.3-2.7 ppm, 3H, m; δ=0.9 ppm, 0.75H, d, J=6.5 Hz, CH_3; δ=0.6 ppm, 2.25H, d, J=6.5 Hz, CH_3.

entry 10: 9l, Yield 69%; m.p. 99-100°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.07 ppm, 0.5H, s, ArOH; δ=12.05 ppm, 0.5H, s, ArOH; δ=11.54 ppm, 1H, 2 x s, ArOH; δ=7.22 ppm, 2H, m, ArH; δ=4.36 ppm, 2H, m, 2 HCO; δ=3.40-3.69 ppm, 3H, m; δ=3.31 ppm, 3H, s, OCH_3; δ=3.16 ppm, 2H, t, J=5 Hz, OCH_2; δ=2.28-2.97 ppm, 3H, m; δ=1.08-1.17 ppm, 2H, m; CH_2; δ=0.57 ppm, 1.48H, d, J=6.3 Hz, CH_3; δ=0.46 ppm, 1.52H, d, J=6.3 Hz, CH_3.

entry 11: 9m, Yield 55%; ^{1}H-NMR (90 MHz, $CDCl_3$): δ=12.3 ppm, 0.46H, s, ArOH; δ=12.2 ppm, 0.54H, s, ArOH; δ=11.7 ppm, 1H, s, ArOH; δ=7.3 ppm, 5H, s, ArH; δ=7.0 ppm, 2H, m, ArH; δ=4.3 ppm, 1H, m, HCO; δ=3.4-3.8 ppm, 3H, m + 1H, qd, J=7.3 Hz and J=3 Hz, CH; δ=3.0-3.3 ppm, 2H, d, J=3 Hz, CH_2; δ=2.3-3.0 ppm, 3H, m; δ=0.9 ppm, 1.62H, d, J=7.3 Hz, CH_3; δ=0.7 ppm, 1.38H, d, J=7.3 Hz, CH_3.

entry 12: 9n, Yield 54%; m.p. 163°C; m/e: 409 (M^+), 258, 165, 151, 135; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.11 ppm, 0.96H, s, ArOH; δ=11.86 ppm, 0.04H, s, ArOH; δ=11.58 ppm, 1H, s, ArOH; δ=7.26 ppm, 2H, s, ArH; δ=6.72-7.03 ppm, 4H, d, J=7.5 Hz and J=8.8 Hz, ArH; δ=4.30 ppm, 1H, m, HCO; δ=3.90 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.77 ppm, 3H, s, OCH_3; δ=3.36-3.67 ppm, 3H, m; δ=2.47 ppm, 1H, d, J=7.8 Hz; δ=2.33 ppm, 2H, dd, J=5.3 Hz; δ=1.03 ppm, 0.12H, d, J=6.3 Hz, CH_3; δ=0.76 ppm, 2.88H, d, J=6.3 Hz, CH_3.

entry 13: 9o, Yield 50%; m.p. 116°C; m/e: 394 (M^+), 276, 256, 119; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.12 ppm, 0.82H, s, ArOH; δ=11.89 ppm, 0.18H, s, ArOH; δ=11.60 ppm, 1H, s, ArOH; δ=7.26 ppm, 2H, s, ArH; δ=6.86-7.13 ppm, 4H, d, J=8.8 Hz, ArH; δ=4.33 ppm, 1H, m, HCO; δ=3.92 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.38-3.69 ppm,3H, m; δ=2.52 ppm, 1H, m; δ=2.32-2.42 ppm, 5H, m p-CH_3 and 2H; δ=1.04 ppm, 0.54H, d, J=6.3 Hz, CH_3; δ=0.74 ppm, 2.46H, d, J=6.3 Hz, CH_3.

entry 14: 9p, Yield 55%; m.p. 126°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.03 ppm, 0.88H, s, ArOH; δ=11.81 ppm, 0.12H, s, ArOH; δ=11.49 ppm, 1H, s, ArOH; δ=7.08-7.28 ppm, 1H, ArH, d, J=7 Hz and 4H, ArH; δ=6.86 ppm, 1H, d, J=7 Hz, ArH; δ=4.25-4.39 ppm, 1H, m, HCO; δ=3.97 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.36-3.78 ppm, 3H; m; δ=2.42 ppm, 3H, m; δ=1.04 ppm, 0.24H, d, J=6.3 Hz, CH_3; δ=0.75 ppm, 2.76H, d, J=6.3 Hz, CH_3.

entry 15: 9q, Yield 51%; m.p. 114-116°C; m/e: 448 (M^+), 276, 258, 231, 175, 173, 105; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.08 ppm, 0.78H, s, ArOH; δ=11.86 ppm, 0.22H, s, ArOH; δ=11.51 ppm, 1H, s, ArOH; δ=7.53 ppm, 2H, dd, J=8 Hz, ArH; δ=7.19-7.41 ppm, 2H, dd, ArH; δ=7.08 ppm, 2H, dd, J=8 Hz, ArH; δ=4.40 ppm, 1H, m, HCO; δ=4.13 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.42-3.78 ppm, 3H, m; δ=2.56 ppm, 1H, dd, J=3 Hz; δ=2.26-2.48 ppm, 2H, dd, J=3 Hz and J=6Hz; δ=1.11 ppm, 0.64H, d, J=6.3 Hz, CH_3; δ=0.82 ppm, 2.36H, d, J=6.3 Hz, CH_3.

entry 16: 9r, Yield 78%; m.p. 154-155°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=12.60 ppm, 0.78H, s, ArOH; δ=12.38 ppm, 0.22H, s, ArOH; δ=12.06 ppm, 1H, s, ArOH; δ=8.04 ppm, 2H, d, J=8Hz, ArH; δ=7.78 ppm, 2H, dd, ArH; δ=7.56 ppm, 2H, d, J=8Hz, ArH; δ=4.43 ppm, 1H, m, HCO; δ=3.94 ppm, 1H, q, J=7 Hz, HCO; δ=3.39-3.78 ppm, 3H, m; δ=2.31-2.58 ppm, 3H, m; δ=1.22 ppm, 2H, m, CH_2; δ=0.61 ppm, 0.66H, t, J=7 Hz, CH_3; δ=0.26 ppm, 2.34H, t, J=7 Hz, CH_3.

entry 5: 16g, Yield 53%; m.p. 114°C; m/e: 432 (M^+), 259, 244, 215, 173; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.08-8.26 ppm, 2H, m, ArH; δ=7.81-7.71 ppm, 2H, m, ArH; δ=7.20 ppm, 5H, s, ArH; δ=4.32 ppm, 1H, m, HCO; δ=3.89 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.44-3.68 ppm, 3H, m; δ=2.44 ppm, 1H, dd, J=7 Hz; δ=2.32 ppm, 2H, d, J=3 Hz; δ=0.94 ppm, 0.15H, d, J=6.3 Hz, CH_3; δ=0.58 ppm, 2.85H, d, J=6.3 Hz, CH_3.

entry 12: 16n, Yield 60%; m.p. 104-116°C; m/e: 378 (M^+), 224, 196, 181, 151, 135; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.03-8.26 ppm, 2H, m, ArH; δ=7.59-7.72 ppm, 2H, m, ArH; δ=6.87 ppm, 2H, dd, J=7 Hz and J=8.6 Hz, ArH; δ=6.59 ppm, 2H, dd, J=7 Hz and J=8.6 Hz, ArH; δ=4.32 ppm, 1H, m, HCO; δ=3.84 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.69 ppm, 3H, s, OCH_3; δ=3.43-3.59 ppm, 3H, m; δ=2.44 ppm, 1H, dd, J=7 Hz; δ=2.29 ppm, 2H, d, J=3 Hz; δ=0.92 ppm, 0.35H, d, J=6.3 Hz, CH_3; δ=0.56 ppm, 2.65H, d, J=6.3 Hz, CH_3.

entry 13: 16o, Yield 50%; m.p. 107°C; m/e: 362 (M^+), 224, 196, 139, 119; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.06-8.27 ppm, 2H, m, ArH; δ=7.67-7.85 ppm, 2H, m, ArH; δ=6.81-7.12 ppm, 4H,

2dd, J=8 Hz, ArH; δ=4.31 ppm, 1H, m, HCO; δ=3.84 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.43-3.75 ppm, 3H, m; δ=2.47 ppm, 1H, dd, J=7 Hz; δ=2.22-2.38 ppm, 5H, m, *p*-CH_3 and 2H; δ=0.92 ppm, 0.24H, d, J=6.3 Hz, CH_3; δ=0.56 ppm, 2.76H, d, J=6.3 Hz, CH_3.

entry 14: 16p, Yield 19%; m.p. 114-120°C; m/e: 381 (M^+), 224, 196, 155, 141; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.04-8.27 ppm, 2H, m, ArH; δ=7.67-7.89 ppm, 2H, m, ArH; δ=7.22 ppm, 2H, d, J=8 Hz, ArH; δ=6.89 ppm, 2H, d, J=8 Hz, ArH; δ=4.37 ppm, 1H, m, HCO; δ=3.93 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.41-3.76 ppm, 3H, m; δ=2.52 ppm, 1H, dd; δ=2.24-2.46 ppm, 2H, dd, J=5 Hz and J=7 Hz; δ=0.93 ppm, 0.15H, d, J=6.3 Hz, CH_3; δ=0.47 ppm, 2.85H, d, J=6.3 Hz, CH_3.

entry 15: 16q, Yield 49%; m.p. 110-114°C; m/e: 416 (M^+), 224, 196, 181, 145, 127; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.10 ppm, 2H, m, ArH; δ=7.78 ppm, 2H, m, ArH; δ=7.49 ppm, 2H, dd, J=8 Hz, ArH; δ=7.04 ppm, 2H, dd, J=8 Hz, ArH; δ=4.40 ppm, 1H, m, HCO; δ=4.08 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.43-3.78 ppm, 3H, m; δ=2.57 ppm, 1H, dd, J=3 Hz and J=7.5 Hz; δ=2.33-2.47 ppm, 2H, dd, J=3 Hz and J=7 Hz; δ=1.00 ppm, 0.54H, d, J=6.3 Hz, CH_3; δ=0.52 ppm, 2.46H, d, J=6.3 Hz, CH_3.

entry 5: 16g, Yield 53%; m.p. 128°C; m/e: 298 (M^+), 194, 176, 148, 121, 105; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.25-7.30 ppm, 3H, m, *o*- and *p*-ArH; δ=7.11 ppm, 2H, dd, J=1.6 Hz and J=8.3 Hz, *m*-ArH; δ=6.96 ppm, 1H, d, J= 10.3 Hz, C=C; δ=6.83 ppm, 1H, d, J= 10.3 Hz, C=; δ=4.43 ppm, 0.05H, q, J=6.3 Hz, HCO; δ=4.26 ppm, 0.95H, m, HCO; δ=4.14 ppm, 0.95H, q, J=6.3 Hz, HCO; δ=4.06 ppm, 0.05H, m, HCO; δ=3.49 ppm, 1H, m; δ=3.35.3.39 ppm, 2H, m; δ=2.27-2.34 ppm, 3H, m; δ=1.19 ppm, 0.15H, d, J=6.3 Hz, CH_3; δ=1.17 ppm, 2.85H, d, J=6.3 Hz, CH_3.

entry 12: 17n, Yield 49%; m.p. 106°C; m/e: 328 (M^+), 176, 147, 135; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.03 ppm, 2H, m, ArH; δ=6.97 ppm, 1H, d, J=11 Hz, C=C; δ=6.84 ppm, 2H, m, ArH and 1H, d, J=11 Hz, C=; δ=4.24 ppm, 1H, m, HCO; δ=4.09 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.78 ppm, 3H, s, OCH_3; δ=3.29-3.50 ppm, 3H, m; δ=2.32 ppm, 1H, dd, J=4 Hz; δ=2.25-2.29 ppm, 2H, dd; δ=1.15 ppm, 3H, d, J=6.3 Hz, CH_3 (only 1 isomer visible in NMR spectrum).

entry 13: 17o, Yield 60%; m.p. 97°C; m/e: 312 (M^+), 176, 149, 135, 121; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.11 ppm, 2H, m, ArH; δ=7.01 ppm, 2H, m, ArH; δ=6.96 ppm, 1H, d, J=10.3 Hz, C=C; δ=6.83 ppm, 1H, d, J=10.3 Hz, C=; δ=4.23 ppm, 1H, m, HCO; δ=4.09 ppm, 1H, q, J=6.3 Hz, HCO; δ=3.47 ppm, 1H, m; δ=3.31-3.39 ppm, 2H, m; δ=2.33 ppm, 1H, dd; δ=2.31 ppm, 3H, s, *p*-CH_3; δ=2.25-2.29 ppm, 2H, dd; δ=1.17 ppm, 0.1H, d, J=6.3 Hz, CH_3; δ=1.15 ppm, 2.9H, d, J=6.3 Hz, CH_3.

entry 14: 17p, Yield 52%; m.p. 97-100°C; m/e: 332 (M^+), 178, 155, 147, 141; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.28 ppm, 2H, d, J=8.3 Hz, ArH; δ=7.03 ppm, 2H, d, J=8.3 Hz, ArH; δ=6.95 ppm, 1H, d, J=10 Hz, C=C; δ=6.81 ppm, 1H, d, J=10 Hz, C=; δ=4.41 ppm, 0.10H, q, J=6.3 Hz, HCO; δ=4.27 ppm, 0.90H, m, HCO; δ=4.15 ppm, 0.90H, q, J=6.3 Hz, HCO; δ=4.05 ppm, 0.10H, m, HCO; δ=3.51 ppm, 1H, m; δ=3.35 ppm, 2H, m; δ=2.32 ppm, 3H, m; δ=1.15 ppm, 3H, d, J=6.3 Hz, CH_3.

entry 15: 17q, Yield 55%; m.p. 105°C; m/e: 366 (M^+), 189, 176, 153, 147, 133; ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.58 ppm, 2H, d, J=8 Hz, ArH; δ=7.24 ppm, 2H, d, J=8 Hz, ArH; δ=6.99 ppm, 2H, d, J=11.4 Hz, C=C; δ=6.84 ppm, 2H, d, J=11.4 Hz, C=; δ=4.50 ppm, 0.18H, q, J=6.3 Hz, HCO; δ=4.26 ppm, 0.82H, q, J=6.3 Hz, HCO; δ=4.33 ppm, 0.82H, m, HCO; δ=4.09 ppm, 0.18H, m, HCO; δ=3.53 ppm, 1H, m; δ=3.39 ppm, 2H, m; δ=2.43 ppm, 1H, dd; δ=2.29-2.39 ppm, 2H, dd; δ=0.99 ppm, 3H, d, J=6.3 Hz, CH_3.

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4.7 References and Notes.

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10 ^{1}H-NMR of the cycloadducts **5**-**7** shows only the *endo* isomer for R = *tert.* butyl (see also reference 7). Therefore, the assumption has been made that only the *endo* adducts are formed (see also references 4a and 4b). The d.e. of the cycloadducts (**8**, **9** and **13**) can be determined by ^{1}H-NMR using the racemic dienes. In three cycloadditions (dienophiles **1**, **2** and **12**), we have also used the optically active diene **4g** made from the (R)-(+)- and (S)-(-)-1-phenylethanol.

11 400 Mhz ^{1}H-NMR was used in most examples. The d.e. was determined by integration of the aromatic OH protons at 12-14 ppm that have different shifts for both diastereomers. The alkyl substituent (R_4) at ~1 ppm also show sufficient shift differences to determine the d.e.

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CHAPTER 5

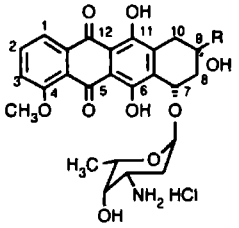
TOTAL SYNTHESIS OF C_{13},C_{14}-ETHYNYL DERIVATIVES OF DAUNOMYCIN AND 4-DEMETHOXYDAUNOMYCIN.

5.1 Introduction.

With the synthetic methods developed for anthracyclinones (see Chapters 2 and 3), we have the possibility of synthesizing anthracyclines with the acetyl group replaced by a C_{13},C_{14}-ethynyl functionality (Figure 5.1).

To our knowledge, no such modifications have as yet been investigated. Although modifications of the C_{13} acetyl or hydroxy acetyl groups (Figure 5.1) were shown to be of little use (see Section 7.4.1), we nevertheless decided to study some ethynyl analogs. There are good reasons to expect that this approach could lead to drugs which have reduced cardiotoxicity (see Chapter 8, section 8.2).

Figure 5.1



1a: Daunomycin R= $-C(=O)CH_3$

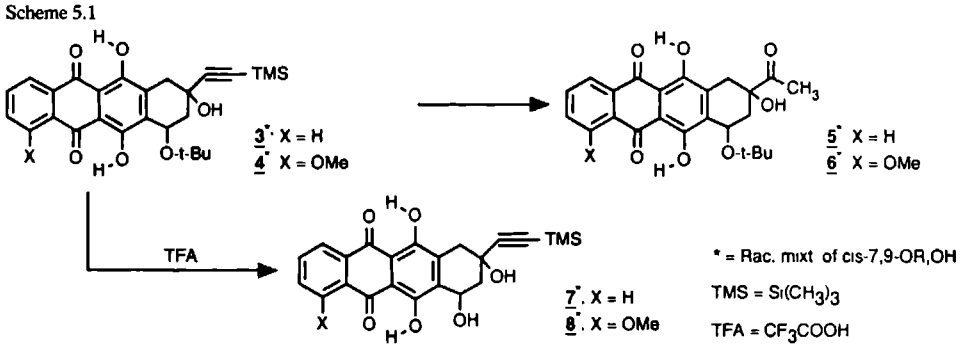
1b: Adriamycin R= $-C(=O)CH_2OH$

2: R = C≡CH

5.2 Synthesis of 9-ethynyl-9-deacetyl derivatives of daunomycin.

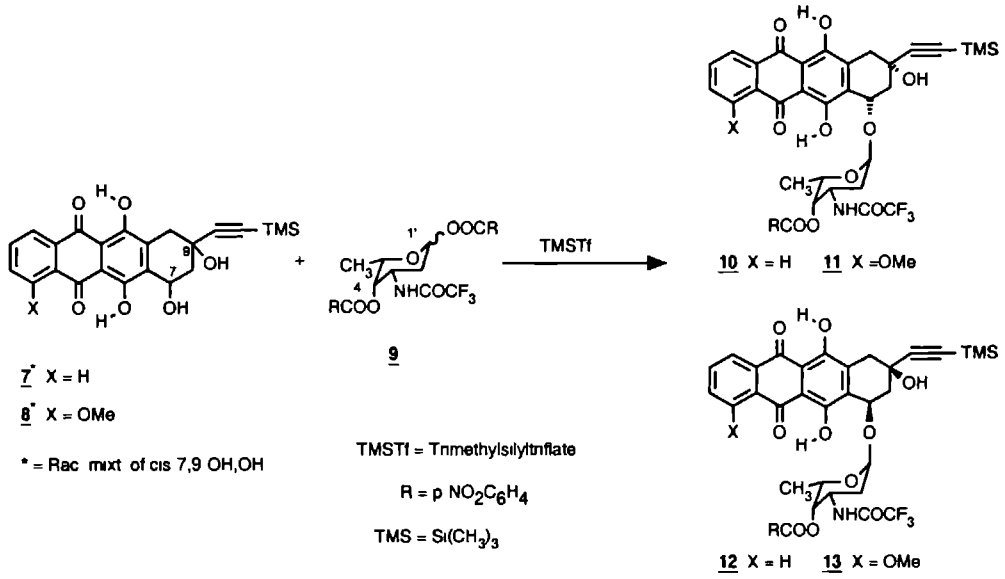
Synthesis of the ethynyl derivatives **2** of daunomycin or 4-demethoxydaunomycin can be achieved by omitting the hydrolysis of the trimethylsilylethynyl group to the acetyl functionality as described in Chapter 2 (conversion **3**/**4** to **5**/**6**, Scheme 5.1).

Scheme 5.1



After removal of the *tert*-butyl group with trifluoroacetic acid from **3** and **4**, the method of Terashima *et al.*[3] was applied to synthesize the corresponding glycosidation products **10**/**11** and **12**/**13** from the compounds **7** and **8** (Scheme 5.2). This method yields exclusively the desired α-glycosides. Under standard conditions however, considerable amounts of diglycoside (having an additional sugar molecule coupled to position 9) were formed. Diastereomers **10**/**12** and **11**/**13** were formed in almost equal amounts. Unfortunately, the diglycosides of the biologically active isomers (**10**/**11**) were formed more rapidly than the diglycosides of the other isomers (**12**/**13**). When the reaction time was prolonged, only the diglycoside of the biologically active isomer was found, while very little of the diglycoside of the other isomer was formed. This observation has also been reported by Irvine *et al.*[4]. We adjusted the conditions to prevent formation of the diglycoside. Lowering the temperature to -25°C decreased formation of the diglycoside but also made the reaction slower.

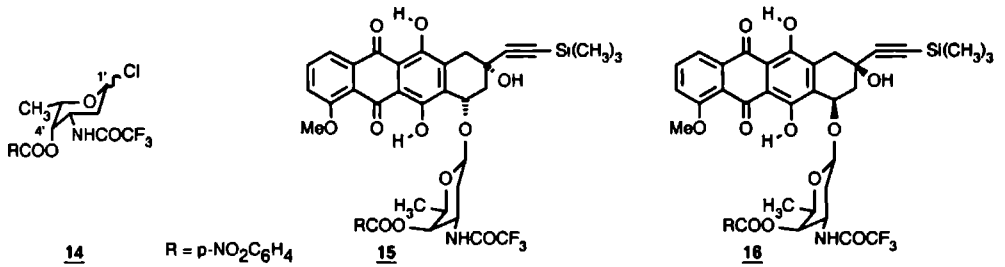
Scheme 5 2



Separation of the diastereomers **10**/**12** and **11**/**13** was very difficult but, could be achieved using column chromatography. The isolated yields of **10** and **12** were not very high (25% and 15%, respectively) because of the problems with this separation. For the methoxy compounds **11** and **13**, the yields were even lower (8% and 12%, respectively). The formation of the diglycoside was competitive with the formation of the mono-glycoside. Separation also appeared to be more troublesome. R_f values were very similar in almost all tested solvent combinations. Therefore, the Koenings-Knorr method was also used for the glycosidation of **8**. This method requires large amounts of the chloro sugar **14** (Figure 5.2), $HgBr_2$ and $Hg(CN)_2$ while the reaction time had to be prolonged to several days and the reaction temperature was increased to 55°C. No diglycosides were formed but this method also yielded unwanted β-glycosides **15** and **16** (Figure 5.2). Although separation of the four isomers **11**, **13**, **15** and **16** was very difficult, it was

possible to purify compound 11 (isolated yield 30%); the other compounds were isolated as a mixture. No further purification of this mixture of isomers was attempted.

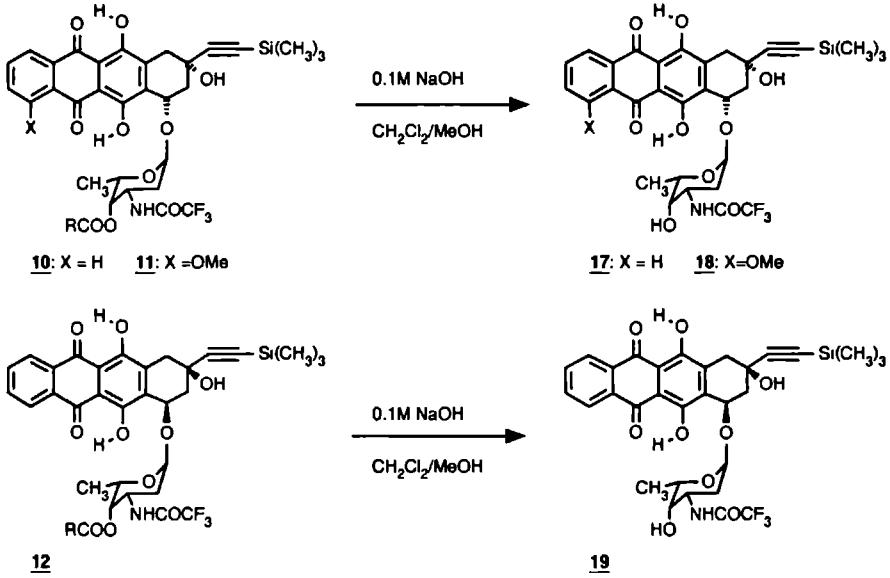
Figure 5.2



The assignment of the structure to both pairs of diastereomers 10/12 and 11/13 was based on ^{1}H-NMR spectral data (shift differences and coupling constants) and optical rotations.

All four compounds 10 - 13 were deprotected at the 4' position. The *para*-nitrobenzoyl group at the 4' position was removed by treatment with a solution of 0.1M sodium hydroxide in a mixture of dichloromethane and methanol. Compounds 17, 18 and 19 were formed in yields between 75 and 80% (Scheme 5.3). Compound 13 was not deprotected because, in this case, we were only interested in the biologically active derivative. Deprotection of 12 to 19 was necessary for confirmation of the structural assignment.

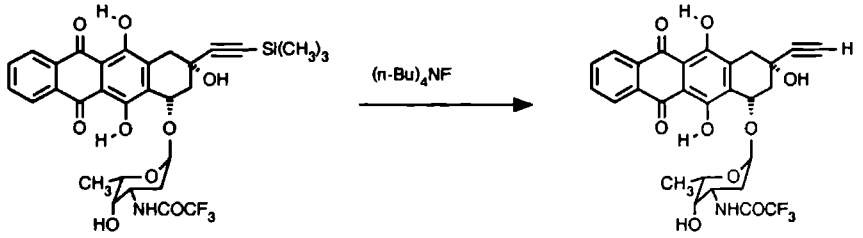
Scheme 5.3



Removal of the trimethylsilyl group from the acetylene function (17) was also possible by treatment with tetrabutylammonium fluoride (Scheme 5.4). Our first intention was to synthesize

both the ethynyl and 1-trimethylsilylethynyl derivatives of daunomycin and 4-demethoxy-daunomycin. Unfortunately, deprotection of the 3' nitrogen was not possible without concomitant removal of the trimethylsilyl group from the acetylene.

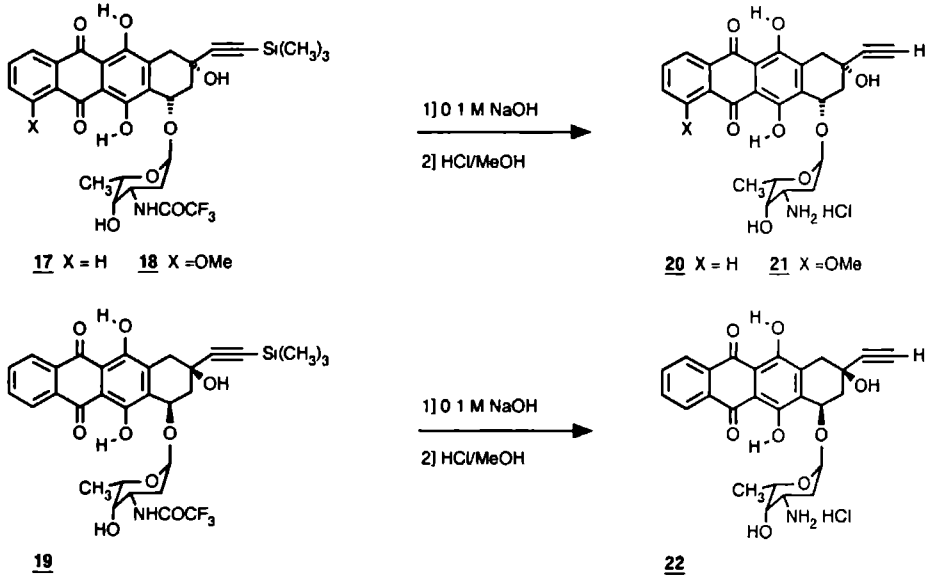
Scheme 5 4



17 17a

By deprotection with 0.1 M sodium hydroxide, both the trifluoroacetyl (at the 2' position) and the trimethylsilyl group (C_{14} position) were removed and HCl addition salts 20/22 and 21 were formed (Scheme 5.5). Compounds 20 and 21 were tested in vitro for biological activity and the results of these tests are described in Chapter 8.

Scheme 5.5



17 X = H 18 X =OMe

20 X = H 21 X =OMe

19 22

5.3 Conclusions.

In this chapter, the synthesis of the C_{13},C_{14}-ethynyl analogs of daunomycin and 4-demethoxy-daunomycin has been described. Intermediates from the synthesis of daunomycinone and 4-demethoxydaunomycinone, as described in Chapter 2, were used as starting materials in the

synthesis of these new analogs. After removal of the *tert*-butoxy protection group the aglycones (7 and 8) were coupled with the chiral protected sugar L-daunosamine (9) according to the method of Terashima and colleagues[3]. It was possible to separate both pairs of diastereomers using column chromatography. After deprotection of the individual diastereomers, we were able to isolate the chiral ethynyl analogs of daunomycin (20) and 4-demethoxydaunomycin (21)

5.4 Experimental section.

General remarks:
^{1}H-NMR-spectra were measured either on a Bruker WH-90 spectrometer or an Bruker AM-400 spectrometer using $(CH_3)_4Si$ as internal standard. $CDCl_3$ was used as solvent unless otherwise stated. Melting points were taken using a Reichert Thermpan microscope and are uncorrected. Mass spectra were taken using a double focusing VG 7070 E mass spectrometer. For column chromatography either Merck Silicagel 60 or Merck silicagel Art. 9385 (flash chromatography) were used. Dry dichloromethane was distilled from CaH_2; dry diethyl ether and dry THF were distilled from sodium benzophenone ketyl. Obtaining correct elemental analysis for anthracyclines is very difficult. In several cases, one molecule of water was included in the molecule. Also in literature[4] examples have been published that one molecule of dichloromethane was included. Sometimes no analysis is given. Compounds must be carefully and repeatedly crystallized to obtain satisfactory analytical data. We tried to obtain satisfactory analyses for the synthesized compounds. Unfortunately not in all cases we have been successful.

Cis-(+/-)-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-9-trimethylsilylethynyl-5,12-naphthacene-dione (7).

1.25 g (2.6 mmol) of 3[5] were dissolved in 15 ml of trifluoroacetic acid. After 10 minutes, when TLC showed the reaction to be complete, the reaction mixture was quenched with 200 ml of water. The aqueous layer was extracted three times with 75 ml of chloroform. The combined organic layers were washed with water, dried over anhydrous sodium sulfate and then evaporated *in vacuo*. The residue was purified by column chromatography (ethyl acetate/ n-hexane, 3 : 5) to give 0.89 g (81%) of 7. m.p. 208-210°C. ^{1}H-NMR (400 MHz) : δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 2.24 ppm (1H, dd, J=14.5 Hz and J=5.2 Hz, H_8(ax)), δ = 2.65 ppm (1H, dt, J=14.5 Hz, J=2.3 Hz and J=2.2 Hz, H_8(eq)), δ = 2.97 ppm (1H, d, J=18.8 Hz, H_{10}(ax)), δ = 3.48 ppm (1H, dd, J=18.5 Hz and J=1.6 Hz, H_{10}(eq)), δ = 3.49 ppm (1H, 7-OH), δ = 3.75 ppm (1H, br s, 9-OH), δ = 5.24 ppm (1H, br s, H_7), δ = 7.82-8.32 ppm (4H, m, ArH), δ = 13.27 ppm (1H, s, ArOH), δ = 13.57 ppm (1H, s, ArOH).

Cis-4-methoxy-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-9-trimethylsilylethynylnathacene-5,12-dione (8).

0.81 g (1.59 mmol) of 4[5] was dissolved in 15 ml of trifluoroacetic acid. Progress of the reaction was followed by TLC (ethyl acetate/n-hexane, 3 : 5). After 10 minutes the reaction mixture was poured into 250 ml of water and then extracted with chloroform until the chloroform extract showed no orange color. After evaporation *in vacuo* the residue was purified by column chromatography (3% methanol in chloroform) to give 0.55 g (76%) of 8. m.p. 235-237°C; MS (FAB) *m/z*: 452 (M^+); ^{1}H-NMR (400 MHz) : δ = 0.19 ppm (9H, s, $Si(CH_3)_3$), δ = 2.26 ppm (1H, dd, J=14.5 Hz and J=5 Hz, H_8(ax)), δ = 2.76 ppm (1H, dt, J=14.5 Hz, J=2.0 Hz and J= 2.5 Hz), H_8(eq)), δ = 2.96 ppm (1H, d, J=18.6 Hz, H_{10}(ax)), δ = 3.48 ppm (1H, d, J=18.6 Hz and J=2.0 Hz, H_{10}(eq)), δ = 3.54 (1H, s, 9-OH), δ = 3.68 ppm (1H, d, J=4.0 Hz, 7-OH), δ = 4.09 (3H, s, OCH_3), δ = 5.28 ppm (1H, m, H_7), δ = 7.38-8.05 ppm (3H, ArH), δ = 13.29 ppm (1H, s, ArOH), δ = 14.03 ppm (1H, s, ArOH); Anal. calcd. for $C_{24}H_{24}O_7Si$: C, 63.70; H, 5.35. Found: C, 63.51; H, 5.32.

9-Deacetyl-4-demethoxy-4'-O-p-nitrobenzoyl-3'-N-trifluoroacetyl-9-trimethylsilylethynyl-daunomycin (10) and 7,9-bis-epi-9-deacetyl-4-demethoxy-4'-O-p-nitrobenzoyl-3'-N-trifluoro-

acetyl-9-trimethylsilylethynyldaunomycin (**12**).

Under an argon atmosphere, 2.75 ml of trimethylsilyltriflate (14.4 mmol) were added to a stirred suspension of 3.55 g of **9** (6.6 mmol) and 20 g molecular sieves 4Å in 125 ml of dry dichloromethane and 430 ml of dry diethyl ether at -25°C. The mixture was stirred at 0°C for 1 h and then cooled to -20°C. A solution of 2.25 g (5.3 mmol) of **7** dissolved in 250 ml of dry dichloromethane was then added. After being stirred at the same conditions for 3 hours (progress of the reaction was followed by TLC, ethyl acetate/benzene, 1:4), the mixture was poured into 1000 ml of a vigorously stirred solution of saturated $NaHCO_3$. The organic layer was separated and washed with 1000 ml of water and 1000 ml of brine, dried over anhydrous sodium sulfate and then concentrated *in vacuo*. The residue was purified by column chromatography (ethyl acetate/toluene, 1 : 16) to give 1.06 g (25%) of diastereomer **10** and 0.64 g (15%) of diastereomer **12**.

Compound **10**: m.p. 157-159°C, $[\alpha^{20}_D]$ = -18.1° (c=0.105 in dioxane); MS (FAB) *m/z*: 796 (M^+); ^{1}H-NMR (400 MHz) : δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 1.31 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 2.03-2.19 ppm (2H, m, 2-H_2·), δ = 2.33 ppm (1H, dd, J= 14.5 Hz and J= 4.8 Hz, H_8(ax)), δ = 2.64 ppm (1H, br d,J=14.5 Hz, H_8(eq)), δ = 3.02 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.57 ppm (1H, d, J=19 Hz, H_{10}(eq)), δ = 3.76 ppm (1H, br s, 9-OH), δ = 4.48-4.58 ppm (2H, m, H_3· and H_5·), δ = 5.17 ppm (1H, m, H_7), δ = 5.44 ppm (1H, br s, H_4·), δ = 5.68 ppm (1H, m, H_1·), δ = 6.51 ppm (1H, br d, J=7.5 Hz, NH), δ = 7.76-7.83 ppm (2H, m, ArH), δ = 8.22-8.36 ppm (6H, m, ArH), δ = 13.29 ppm (1H, s, ArOH), δ = 13.59 ppm (1H, s, ArOH).

Compound **12**: m.p. 160-162°C, $[\alpha^{20}_D]$ = -310.7° (c=0.112 in dioxane); MS (FAB) *m/z*: 796 (M^+); ^{1}H-NMR (400 MHz) : δ = 0.22 ppm (9H, s, $Si(CH_3)_3$), δ = 1.27 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.92-2.18 ppm (3H, m, 2-H_2· and H_8(ax)), δ = 2.90 ppm (1H, m, H_8(eq)), δ = 3.10 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.70 ppm (1H, d, J=19 Hz, H_{10}(eq)), δ = 4.21 ppm (1H, s, 9-OH), δ = 4.59 ppm (1H, m, H_3·), δ = 4.78 ppm (1H, q, J=6.5 Hz. H_5·), δ = 5.44 ppm (1H, br s, H_7), δ = 5.54 ppm (1H, m, H_4·), δ = 5.58 ppm (1H, br d, J=3 Hz, H_1·), δ = 6.36 ppm (1H, br d, J=7.5 Hz, NH), δ = 7.85-7.89 ppm (2H, m, ArH), δ = 8.25-8.41 ppm (6H, m, ArH), δ = 13.35 ppm (1H, s, ArOH), δ = 13.82 ppm (1H, s, ArOH).

9-Deacetyl-4'-O-p-nitrobenzoyl-3'-N-trifluoroacetyl-9-trimethylsilylethynyldaunomycin (**11**) *and 7,9-bis-epi-9-deacetyl-4'-O-p-nitrobenzoyl-3'-N-trifluoroacetyl-9-trimethylsilylethynyl-daunomycin* (**13**).

1.0 ml of trimethylsilyltriflate was added to a stirred suspension of 1.22 g of **9** (2.26 mmol) and 16 g molecular sieves 4Å in a mixture of 150 ml of dry dichloromethane and 50 ml of dry diethyl ether at -25°C under an argon atmosphere. The whole mixture was stirred at 0°C for 1 h to give a clear solution, which was cooled at -25°C. A solution of 0.8 g (1.8 mmol) of **8** dissolved in 200 ml of dry dichloromethane was then added and the reaction mixture was stirred at -20°C for 3 h. Progress of the reaction was followed by TLC (ethyl acetate/benzene, 1 : 4) and, after all of compound **8** had disappeared, the reaction mixture was poured into 800 ml of saturated $NaHCO_3$ which was vigorously stirred to quench the glycosidation reaction. The organic layer was separated, washed with 500 ml of water and 500 ml of brine and dried over anhydrous sodium sulfate. After evaporation *in vacuo*, compounds **11** and **13** were separated using column chromatography (2% acetone and 0.2% glacial acetic acid in dichloromethane). Both purified samples were triturated with n-hexane to give 120 mg (8%) of **11** and 0.170 g (12%) of **13**.

Compound **11**: m.p. 161-163°C, $[\alpha^{20}_D]$ = +6° (c=0.5 in dioxane); MS (FAB) *m/z*: 826 (M^+); ^{1}H-NMR (400 MHz): δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 1.29 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 2.04 ppm (2H, dd, J=9 Hz and J= 2.3 Hz, 2-H_2·), δ = 2.33 ppm (1H, dd, J=14.7 Hz and J=4.9 Hz, H_8(ax)), δ = 2.61 ppm (1H, m, H_8(eq)), δ = 3.01 ppm (1H, d, J=18.7 Hz, H_{10}(ax)), δ = 3.57 ppm (1H, dd, J=18.7 Hz and J=1.2 Hz, H_{10}(eq)), δ = 3.70 ppm (1H, s, 9-OH), δ = 4.07 ppm (3H, s, OMe), δ = 4.44-4.55 ppm (2H, m, H_3· and H_5·), δ = 5.23 ppm (1H, br s, H_7), δ = 5.46 ppm (1H, m, H_4·), δ = 5.71 ppm (1H, br s, H_1·), δ = 6.22 ppm (1H, br d, J=7.5 Hz, NH), δ = 7.37-8.04 ppm (3H, m, ArH), δ = 8.27-8.36 ppm (4H, m, ArH), δ = 13.29 ppm (1H, s, ArOH), δ = 14.07 ppm (1H, s, ArOH); Anal. calc. for $C_{32}H_{32}F_3N_2O_{14}Si$. 1 H_2O: C, 55.45; H, 4.65; N 3.33 Found: C, 54.72; H, 4.41; N, 3.79.

Compound **13**: m.p. 174-176°C, $[\alpha^{20}_D]$ = -347.7° (c=0.65 in dioxane); MS (FAB) *m/z*: 826 (M^+); ^{1}H-NMR (400 MHz) : δ = 0.22 ppm (9H, s, $Si(CH_3)_3$), δ = 1.25 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.90-2.17 ppm (3H, m, 2-H_2· and H_8(ax)), δ = 2.86-2.92 ppm (1H, m, H_8(eq)), δ =

3.08 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.71 ppm (1H, dd, J=19 Hz and J=1.3 Hz, H_{10}(eq)), δ = 4.11 ppm (3H, s, OMe), δ = 4.23 ppm (1H, s, 9-OH), δ = 4.52-4.57 ppm (1H, m, $H_{3'}$), δ = 4.76-4.84 ppm (1H, m, $H_{5'}$), δ = 5.40 ppm (1H, br s, H_7), δ = 5.56 ppm (1H, br s, $H_{4'}$), δ = 5.57 ppm (1H, br s, $H_{1'}$), δ = 6.27 ppm (1H, br d, J=7.5 Hz, NH), δ = 7.79-8.27 ppm (3H, m, ArH), δ = 8.28-8.35 ppm (4H, m, ArH), δ = 13.31 ppm (1H, s, ArOH), δ = 14.23 ppm (1H, s, ArOH).

9-Deacetyl-4'-O-p-nitrobenzoyl-3'-N-trifluoroacetyl-9-trimethylsilylethynyldaunomycin (**11**).

452 mg (1.0 mmol) of **8** were dissolved in 100 ml of dry THF and 2.08 g (8.24 mmol) of $Hg(CN)_2$, 0.944 g (2.63 mmol) of $HgBr_2$ and 4.8 g molecular sieves 3Å were added. The reaction mixture was stirred under an argon atmosphere at 50-55°C for 2 h. Three 1M portions of freshly prepared chloro sugar **14** were added at 0, 4 and 22 h, while the temperature was maintained at 50-55°C. The chloro sugar **14** was prepared by bubbling anhydrous hydrogen chloride into a suspension of 2,3,6-trideoxy-1,4-di-*p*-O-nitrobenzoyl-3-trifluoroacetamido-α-L-*lyxo*-hexopyranose (**9**, 540 mg, 1.0 mmol) in dry 16 ml of dichloromethane at 0°C for 3 min The mixture was allowed to stand at room temperature for 10 min, filtered to remove the precipitated *p*-nitrobenzoic acid, and evaporated. The residue was dissolved in 8 ml of dry dichloromethane and added to the reaction mixture. Additional 2.08 g (8.24 mmol) of $Hg(CN)_2$, 0.944 g (2.63 mmol) of $HgBr_2$ and 4.8 g molecular sieves 3Å were added at 4 h. The total reaction time after the first addition of **14** was 25 h. The reaction mixture was filtered, the solids were washed with THF and the combined organic layers were evaporated and the residue was triturated with 300 ml of chloroform and filtered. The filtrate was washed twice with a solution of 100 ml 30% KI and once with water. After drying over anhydrous sodium sulfate and evaporation *in vacuo* the residue was purified by column chromatography (silicagel 60H, ethyl acetate/benzene, 1 : 4) to give 248 mg (30%) of **11**. Besides **11** an equal amount of **13** and small amounts of **15** and **16** were isolated.

9-Deacetyl-4-demethoxy-3'-N-trifluoroacetyl-9-trimethylsilylethynyldaunomycin (**17**).

Under an argon atmosphere and at 0°C, 6.5 ml of 0.1M sodium hydroxide were added to a stirred solution of 0.5 g (0.63 mmol) of **10** in 4 ml of dichloromethane and 260 ml of methanol. The purple solution was stirred at room temperature for 5 minutes at 0°C. Glacial acetic acid was added to the reaction mixture until the color of the solution became bright orange. 400 ml of ethyl acetate and 400 ml of brine were added to the reaction mixture. The organic layer was washed twice with 125 ml of brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was purified by column chromatography (dichloromethane/acetone, 9 : 1) to give 0.31 g (76%) of **17**. m.p. 140-142°C, $[\alpha^{20}_D]$ = +237° (c=0.076 in dioxane); MS (FAB) *m/z*: 647 (MH^+); ^{1}H-NMR (400 MHz) : δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 1.34 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.79-2.03 ppm (2H, m, 2-$H_{2'}$), δ = 2.01 ppm (1H, br s, 4'-OH), δ = 2.28 ppm (1H, dd, J=14.7 Hz and J=4.5 Hz, H_8(ax)), δ = 2.62 ppm (1H, m, H_8(eq)), δ = 3.01 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.60 ppm (1H, dd, J=19 Hz and J=1.5 Hz, H_{10}(eq)), δ = 3.64-3.69 ppm (1H, m, $H_{4'}$), δ = 3.92 ppm (1H, s, 9-OH), δ = 4.19-4.26 ppm (1H, m, $H_{3'}$), δ = 4.32-4.38 ppm (1H, q, J=6.5 Hz, $H_{5'}$), δ = 5.19 ppm (1H, dd, J=4 Hz and J=2 Hz, H_7), δ = 5.53 ppm (1H, br d, J=4 Hz, $H_{1'}$), δ = 6.65 ppm (1H, br d, J=8 Hz, NH), δ = 7.82-7.86 ppm (2H, m, ArH), δ = 8.33-8.38 ppm (2H, m, ArH), δ = 13.35 ppm (1H, s, ArOH), δ = 13.63 ppm (1H, s, ArOH).

9-Deacetyl-4-demethoxy-9-ethynyl-3'-N-trifluoroacetyl-daunomycin (**17a**).

178 mg tetrabutylammonium fluoride were added to a solution of 244 mg of **17** in 25 ml of THF. The mixture was stirred at room temperature under an argon atmosphere for 5 minutes. 100 ml of water and 100 ml of chloroform were added to the reaction mixture and the aqueous layer was extracted twice with 100 ml of chloroform. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was purified by column chromatography (dichloromethane/acetone, 7 : 3) to give 130 mg of **17a** (60%). m.p. 146-148°C, $[\alpha^{20}_D]$ = +289° (c=0.076 in dioxane); MS (FAB) *m/z*: 575 (M^+); ^{1}H-NMR (400 MHz) : δ = 1.31 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.80-2.08 ppm (3H, m, 2-$H_{2'}$ and H_8(ax)), δ = 2.18 ppm (1H, br s, 4'-OH), δ = 2.57 ppm (1H, s, C≡C-H), δ = 2.87 ppm (1H, m, H_8(eq)), δ = 3.10 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.71 ppm (1H, d, J=19 Hz, H_{10}(eq)), δ = 3.62 ppm (1H, m, $H_{4'}$), δ = 4.30 ppm (1H, s, 9-OH), δ = 4.31 ppm (1H, m, $H_{3'}$), δ = 4.59 ppm

(1H, m, $H_{5'}$), δ = 5.41 ppm (1H, m, H_7), δ = 5.51 ppm (1H, br s, $H_{1'}$), δ = 6.64 ppm (1H, br d, J=8 Hz, NH), δ = 7.83-7.88 ppm (2H, m, ArH), δ = 8.35-8.40 ppm (2H, m, ArH), δ = 13.41 ppm (1H, s, ArOH), δ = 13.79 ppm (1H, s, ArOH); Anal. calc. for $C_{28}H_{24}F_3NO_9$. 1 H_2O: C, 56.66; H, 4.42; N 4.36. Found: C, 57.06; H, 4.45; N, 4.60

9-Deacetyl-3'-N-trifluoroacetyl-9-trimethylsilylethynyldaunomycin (**18**).

Sodium hydroxide (0.1M, 3.3 ml) was added to a stirring solution of 248 mg (0.3 mmol) of **11** in 3 ml of dichloromethane and 100 ml of methanol at 0°C under an argon atmosphere. The deep purple solution was stirred at 0°C for 3 minutes. Glacial acetic acid was added to the reaction mixture until the color of the solution became bright orange. 150 ml of ethyl acetate and 150 ml of brine were added to the reaction mixture. The organic layer was washed twice with 75 ml of brine and dried over anhydrous sodium sulfate. Filtration and concentration *in vacuo* gave an orange residue, which was purified by column chromatography (chloroform/methanol/ glacial acetic acid, 100 : 1 : 0,5) to give 156 mg (77%) of **18**. m.p. 159-163°C; $[\alpha^{20}_D]$ = +290° (c=0.07 in dioxane); ^{1}H-NMR (400 MHz) : δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 1.33 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.78-2.09 ppm (2H, m, 4'-OH and 2-$H_{2'}$), δ = 2.28 ppm (1H, dd, J=14.7 Hz and J=4.5 Hz, H_8(ax)), δ = 2.60 ppm (1H, m, H_8(eq)), δ = 2.99 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.59 ppm (1H, dd, J=19 Hz and J=1.5 Hz, H_{10}(eq)), δ = 3.63-3.67 ppm (1H, m, $H_{4'}$), δ = 3.90 ppm (1H, s, 9-OH), δ = 4.08 ppm (3H, s, OMe), δ = 4.20 ppm (1H, m, $H_{3'}$), δ = 4.33 ppm (1H, q, J=6.5 Hz, $H_{5'}$), δ = 5.21 ppm (1H, m, H_7), δ = 5.54 ppm (1H, br d, J=3.8 Hz, $H_{1'}$), δ = 6.58 ppm (1H, br d, J=8.5 Hz, NH), δ = 7.38-8.06 ppm (3H, m, ArH), δ = 13.32 ppm (1H, s, ArOH), δ = 14.04 ppm (1H, s, ArOH); Anal. calc. for $C_{32}H_{36}F_3NO_{11}Si$. 1 H_2O: C, 55.25; H, 5.22; N 2.01. Found: C, 55.24; H, 5.07; N, 2.12.

7,9-*Bis-epi*-9-*deacetyl*-4-*demethoxy*-3'-*N*-*trifluoroacetyl*-9-*trimethylsilylethynyldaunomycin* (**19**).

At 0°C and under an argon atmosphere 11.8 ml of a 0.1M sodiumhydroxide solution was added to a solution of 0.9 g (1,13 mmol) of **12** in 7 ml of dichloromethane and 475 ml of methanol. The deep purple solution was stirred at 0°C for 20 minutes and progress of the reaction was monitored by TLC (dichloromethane : acetone, 9 : 1). Glacial acetic acid was added to the mixture until the colour of the solution became orange. Ethylacetate (750 ml) and brine (750 ml) were added to the reaction mixture. The organic layer was washed with twice with 200 ml of brine and dried over anhydrous sodiumsulfate. After filtration and evaporation *in vacuo* the orange residue was purified by column chromatography (dichloromethane : acetone, 9 : 1) to give 0.58 g of **19** (80%). m.p. 138-140°C, $[\alpha^{20}_D]$ = -375° (c=0.0885 in dioxane). ^{1}H-NMR (400 MHz) : δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 1.31 ppm (3H, d, J=6.5 Hz, 5'-Me), δ = 1.82-1.95 ppm (3H, m, 2-$H_{2'}$ and 4'-OH), δ = 2.01 ppm (1H, dd, J=14.7 Hz and J=3.5 Hz, H_8(ax)), δ = 2.86 ppm (1H, d, J=14.7 Hz, H_8(eq)), δ = 3.09 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.60-3.64 ppm (1H, m, $H_{4'}$), δ = 3.70 ppm (1H, d, J=19 Hz , H_{10}(eq)), δ = 4.23 ppm (1H, br s, 9-OH), δ = 4.25-4.30 ppm (1H, m, $H_{3'}$), δ = 4.57 ppm (1H, q, J=6.5 Hz, $H_{5'}$), δ = 5.41 ppm (1H, br d, J=4 Hz, H_7), δ = 5.48 ppm (1H, s, $H_{1'}$), δ = 6.64 ppm (1H, br d, J=8 Hz, NH), δ = 7.84-7.88 ppm (2H, m, ArH), δ = 8.35-8.39 ppm (2H, m, ArH), δ = 13.37 ppm (1H, s, ArOH), δ = 13.79 ppm (1H, s, ArOH); Anal. calc. for $C_{31}H_{32}F_3NO_9Si$. 1 H_2O: C, 55.93; H, 5.15; N 2.10. Found: C, 55.71; H, 4.92; N, 2.39.

9-Deacetyl-4-demethoxy-9-ethynyl-daunomycin (**20**).

30 ml of 0.1M sodium hydroxide were added to a solution of 0.16 g (0.23 mmol) of **17** in 3 ml of acetone. The reaction mixture was stirred at room temperature for 30 minutes under an argon atmosphere. Progress of the reaction was monitored by TLC (water/ acetic acid/ methanol/ chloroform, 12 : 26 : 54 : 160). The pH of the reaction mixture was adjusted to 9 with 1M hydrogen chloride solution and extracted with chloroform (approximately 5 times) until a chloroform extract showed no orange color of the reaction product. The combined organic layers were washed with water and dried over anhydrous sodium sulfate. After filtration and evaporation *in vacuo*, the residue was dissolved in a minimal amount of chloroform/methanol (9 : 1) and 0.4 ml of 0.6M hydrogen chloride in methanol and 50 ml of dry diethyl ether were added. The orange powder that precipitated was filtered off to give 84 mg (70%) of **20**. m.p.

177-179°C, $[\alpha^{20}_D]$ = +189° (c=0.037 in dioxane); MS (FAB) *m/z*: 633 (M^+); ^{1}H-NMR (400 MHz, DMSO) : δ = 1.16 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.74 ppm (1H, d, J= 9 Hz, H_2·(ax)), δ = 1.90 ppm (1H, t, J=12 Hz, H_2·(eq)), δ = 2.13 ppm (1H, dd, J=13 Hz and J=6.5 Hz, H_8(ax)), δ = 2.43 ppm (1H, d, J=13 Hz, H_8(eq)), δ = 2.5 ppm (1H, s, C≡C-H), δ = 2.91 ppm (1H, d, J=18 Hz, H_{10}(ax)), δ = 3.08 ppm (1H, d, J=18 Hz, H_{10}(eq)), δ = 3.42 ppm (1H, m, H_3·), δ = 3.59 ppm (1H, br s, H_4·), δ = 4.03 ppm (1H, q, J=6.5 Hz, H_5·), δ = 5.41 ppm (1H, m, H_7), δ = 5.32 ppm (1H, br s, H_1·), δ = 5.47 ppm (1H, br s, 4'-OH), δ = 5.95 ppm (1H, s, 9-OH), δ = 7.96 ppm (3H, br s, 3'-NH_3), δ = 7.93-7.96 ppm (2H, m, ArH), δ = 8.21-8.24 ppm (2H, m, ArH), δ = 13.23 ppm (1H, s, ArOH), δ = 13.53 ppm (1H, s, ArOH).

9-Deacetyl-9-ethynyl-daunomycin (21).

156 mg (0.23 mmol) of 18 were dissolved in a minimal amount of acetone. Sodium hydroxide (0.1M, 26 ml) was then added and the reaction mixture was stirred at room temperature under an argon atmosphere for 30 minutes. Progress of the reaction was followed by TLC (water/Glacial acetic acid/Methanol/Chloroform, 12 : 26 : 54 : 160). After the pH of the reaction mixture had been adjusted to 9 with 1M hydrogen chloride, the solution was extracted with 100 ml of chloroform until a chloroform extract showed no orange color of the reaction product. The combined organic layers were washed with water and dried over anhydrous sodium sulfate. After filtration and evaporation *in vacuo*, the residue was dissolved in a minimal amount of chloroform/methanol (9/1). After adding 0.7 ml of 0.6M hydrogen chloride in methanol and 100 ml of diethyl ether were added, the HCl salt 21 (98 mg, 78%) was precipitated as an orange powder. m.p. 227-230°C, $[\alpha^{20}_D]$ = +93° (c=0.0475 in dioxane); ^{1}H-NMR (400 MHz, DMSO) : δ = 1.16 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.65-1.91 ppm (2H, m, 2 H_2·), δ = 2.13 ppm (3H, dd, J=13 Hz and J=6.5 Hz, H_8(ax)), δ = 2.35 ppm (1H, d, J=13 Hz, H_8(eq)), δ = 2.66 ppm (1H, s, C≡C-H), δ = 2.88 ppm (1H, d, J=18 Hz, H_{10}(ax)), δ = 3.07 ppm (1H, d, J=18 Hz, H_{10}(eq)), δ = 3.34 ppm (1H, m, H_3·), δ = 3.56 ppm (1H, br s, H_4·), δ = 3.98 ppm (3H, s, OCH_3·), δ = 4.08 ppm (1H, q, J=6.5 Hz, H_5·), δ = 4.98 ppm (1H, m, H_7), δ = 5.30 ppm (1H, br s, H_1·), δ = 5.47 ppm (1H, br s, 4'-OH), δ = 5.95 ppm (1H, s, 9-OH), δ = 7.63-7.66 ppm (1H, m, ArH), δ = 7.86-7.89 ppm (2H, m, ArH), δ = 7.90 ppm (3H, br s, 3'-NH_3), δ = 13.20 ppm (1H, s, ArOH), δ = 14.06 ppm (1H, s, ArOH).

7,9-Bis-epi-9-deacetyl-4-demethoxy-9-ethynyl-daunomycin (22).

250 mg (0.39 mmol) of compound 19 were dissolved in 5 ml of acetone. 50 ml of 0.1M sodium hydroxide solution were added and the reaction mixture was stirred for 30 minutes at room temperature under an argon atmosphere. Progress of the reaction was monitored by TLC (water/acetic acid/methanol/chloroform, 12 : 26 : 54 : 160). After the pH of the reaction mixture had been adjusted to 9 with 1M hydrogen chloride, the solution was extracted with 100 ml of chloroform until the chloroform extract showed no orange color of the reaction product. The combined organic layers were washed with water and dried over anhydrous sodium sulfate. After filtration and evaporation *in vacuo* the residue was dissolved in a minimal amount of a mixture of chloroform/methanol (9/1). After adding 0.7 ml of 0.6M hydrogen chloride in methanol and 100 ml of diethyl ether, the HCl salt of 22 (140 mg, 70%) precipitated as an orange powder. m.p. 160-162°C, $[\alpha^{20}_D]$ = -280° (c=0.0275 in dioxane); MS (FAB) *m/z*: 480 (M^+-Cl); ^{1}H-NMR (400 MHz, DMSO) : δ = 1.14 ppm (3H, d, J=6.5 Hz, 6'-Me), δ = 1.60-1.95 ppm (2H, m, H_2·(ax) and H_2·(eq), δ = 2.14 ppm (1H, dd, J=14 Hz and J=5 Hz, H_8(ax))δ = 2.43 ppm (1H, d, J=13 Hz, H_8(eq)), δ = 2.66 ppm (1H, s, C≡C-H), δ = 2.98 ppm (1H, d, J=18 Hz, H_{10}(ax)), δ = 3.07 ppm (1H, d, J=18 Hz, H_{10}(eq)), δ = 3.42 ppm (1H, m, H_3·), δ = 3.59 ppm (1H, br s, H_4·), δ = 4.00 ppm (1H, q, J=6.5 Hz, H_5·), δ = 5.26 ppm (1H, m, H_7), δ = 5.24 ppm (1H, br s, H_1·), δ = 5.80 ppm (1H, br s, 4'-OH), δ = 6.31 ppm (1H, s, 9-OH), δ = 7.83 ppm (3H, br s, 3'-NH_3), δ = 7.96-7.99 ppm (2H, m, ArH), δ = 8.27-8.29 ppm (2H, m, ArH), δ = 13.27 ppm (1H, s, ArOH), δ = 13.49 ppm (1H, s, ArOH).

5.5 References.

1 Arcamone, F., Anticancer Agents based on Natural Products Models, Medicinal Chemistry Volume 16, Ed. Cassady, J.M. and Dourous J.D., Academic Press, **1980**, 1-41.

2 Weiss, R.B., *Seminar in Oncology*, **1992**, *19(6)*, 670.
3 Kimura, Y., Suzuki, M., Matsumoto T., Abe, R. and Terashima, S., *Bull. Chem. Soc. Japan*, **1986**, *59*, 423.
4 Irvine R.W., Kinloch S.A., McCormick A.S., Russell, R.A. and Warrener R.N., *Tetrahedron*, **1988**, *44(14)*, 4591.
5 For the synthesis of compounds 3 and 4, see Chapter 2, compounds 45 and 27, respectively.

CHAPTER 6

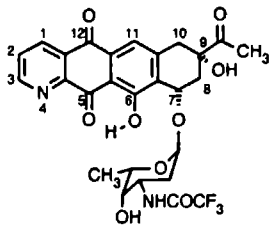
TOTAL SYNTHESIS OF PYRIDINE D RING DERIVATIVES OF DAUNOMYCIN.

6.1 Introduction.

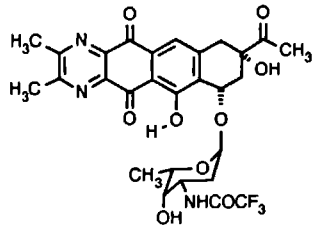
Severe cardiotoxicity is a serious problem for the medical application of anthracycline antibiotics such as daunomycin and adriamycin. The generation of reactive oxygen species such as $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$, has been used to explain the anti-tumor activity of the anthracycline antibiotics. However, it can also be used to explain their cardiotoxicity. Since anthracyclines accumulate in the heart, and cardiac tissue is not able to protect the cell from oxidative lesions, the one-electron reduction of these compounds to their radical anions, which will yield reactive oxygen species, may play an important role in cardiotoxicity[1]. Modifications of the redox potential of the anthracyclinone part (aglycone) may influence both the anti-tumor activity and the cardiotoxicity[2] (see also Chapter 7). This redox potential can most easily be affected by substituents in the D ring of the aglycone. Introduction of a nitrogen atom in the D ring will affect the redox potential. Furthermore, the nitrogen atom provides an extra position for protonation or interaction with an electrophilic receptor molecule.

In 1990 and 1991, Kita *et al.*[3,4] published a synthesis method for D ring pyridine 1 and D ring pyrazine 2 analogs of 11-deoxydaunomycin (Figure 6.1). Although they were able to synthesize the D ring indole 3 derivative of daunomycin, they did not describe the synthesis of the D ring pyridine derivative of daunomycin.

Figure 6.1



1



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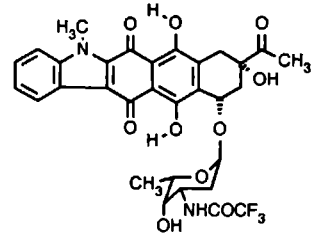


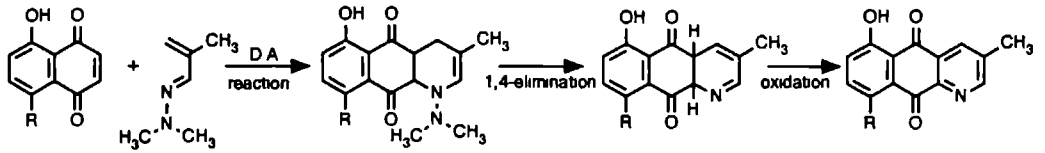
3

Introduction of a nitrogen atom in the D ring is theoretically possible in four positions. We chose to synthesize the 1-N and 4-N derivatives of daunomycin because they may be accessible using 1-aza-buta-1,3-dienes in the Diels-Alder reaction with quinones[5].

6.2 Synthesis of D ring pyridine analogs of daunomycinone.

In Chapter 3 we described the synthesis of the complete ABC fragment of daunomycinone. This fragment, available on a gram scale, was used as starting material for the synthesis of new D ring derivatives of daunomycin. The intended synthesis of pyridine D ring derivatives could be accomplished by Diels-Alder reaction of the ABC fragment with 1-aza-buta-1,3-dienes.
1-(Dimethylamine)-3-methyl-1-aza-buta-1,3-diene **4** has been successfully applied by Potts *et al.*[5] in the Diels-Alder reaction with quinones, such as juglone **5** and naphthazarin **6** to yield aza-anthraquinones **11** and **12** (Scheme 6.1).

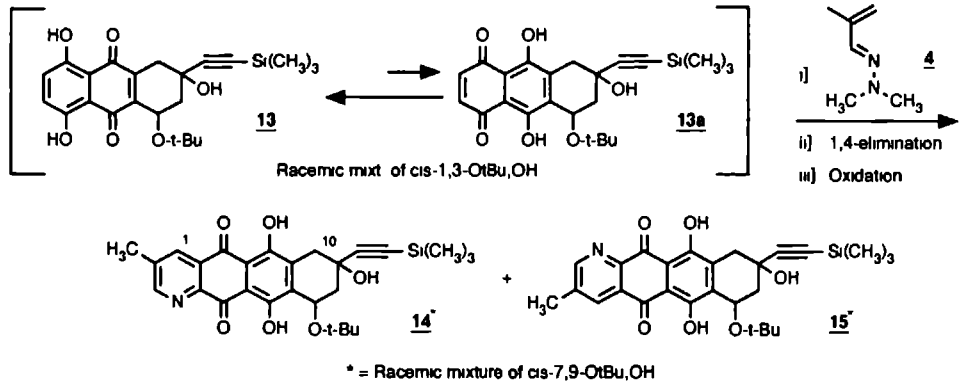
Scheme 6.1



5 R = H, **6** R = OH — **4** — **7** R = H, **8** R = OH — **9** R = H, **10** R = OH — **11** R = H, **12** R = OH

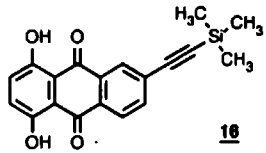
In the cycloaddition of juglone **5**, the azadiene showed high regioselectivity. The strong electron-donating dimethylamino group causes the azadiene to react as an electron-rich diene with the highest HOMO coefficient on the γ-carbon atom. In the Diels-Alder reaction of **4** with naphthazarin **6**, cycloadduct **10**, derived from the 1:1 cycloadduct by loss of dimethylamine, was isolated together with the oxidized product **12** and the addition product of dimethylamine to naphthazarin, 2-(dimethylamino)-5,8-dihydroxy-1,4-naphthoquinone[5]. When argon was bubbled through the reaction mixture at 40-45°C, the dimethylamine was removed and cycloadduct **10** was isolated in a yield of 84%. After air oxidation by heating **10** with potassium carbonate in a methanol/water mixture, cycloadduct **12** was isolated with an overall yield of 77%.
The cycloaddition of azadiene **4** with dienophile **13** (Scheme 6.2) was expected to proceed in a similar way as described for naphthazarin **6** (Scheme 6.1).

Scheme 6.2



In orientating experiments, cycloaddition with the ABC fragment appeared to be much slower at room temperature. Due to the slow conversion, extensive decomposition of the ABC fragment 13, leading to compound 16 (Figure 6.2), was observed, probably caused by elimination of *tert*-butyl alcohol and water from 13 by the basic amines in the reaction mixture.

Figure 6.2



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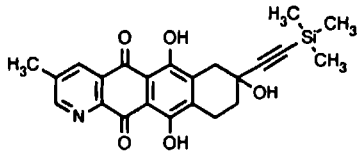
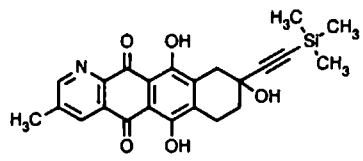
Therefore, we decided to perform the cycloaddition under high pressure (6 - 15 kbar at room temperature). It was expected that, under these conditions, the reaction would be accelerated and that elimination of dimethylamine, which may cause side reactions, would thus be avoided.

The high pressure Diels-Alder reaction of diene 4 with the ABC fragment 13 gave a complex mixture of products. The best results were obtained with dichloromethane as solvent. After evaporation of the solvent *in vacuo*, the ^{1}H-NMR spectrum of the crude reaction mixture (treated with oxygen) showed the presence of pyridine ring containing products 14 and 15 but also other pyridine containing products lacking the *tert*-butoxy group (typical pyridine protons at 8.4 and 8.9 ppm were present). Furthermore, the reaction mixture contained considerable amounts of the salt $(CH_3)_2NH_2^+$ Cl^- which had been formed from dimethylamine and dichloromethane at high pressure[6]. After chromatographic separation, 14 and 15 were obtained pure. A third compound without the *tert*-butoxy group was isolated in a yield of 40%. Probably, a mixture of 17 and 18 (Figure 6.3) had been formed. It was not possible to separate these compounds by chromatography. No conclusions can be drawn from the ^{1}H-NMR spectra, due to the (expected) absence of differences in phenolic hydroxy proton shifts between 17 and 15.

Figure 6.3



17

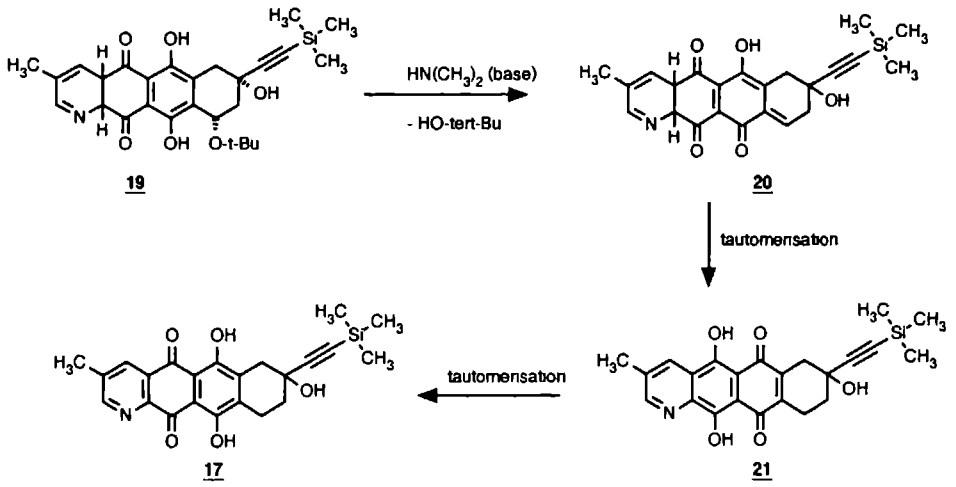


18

This result, which was quite surprising, indicated that, also under high pressure conditions dimethylamine was eliminated from the initial 1:1 cycloadduct. The formation of 17/18 can be explained by the reaction sequence give in Scheme 6.3 (given for compound 17 only).
When the reaction was performed in THF, compounds 17/18 became the main products after evaporation *in vacuo* of the solvent. In dichloromethane, base-catalysed elimination of *tert*-butyl

alcohol was probably retarded because dimethylamine reacts under high pressure with the solvent.

Scheme 6 3

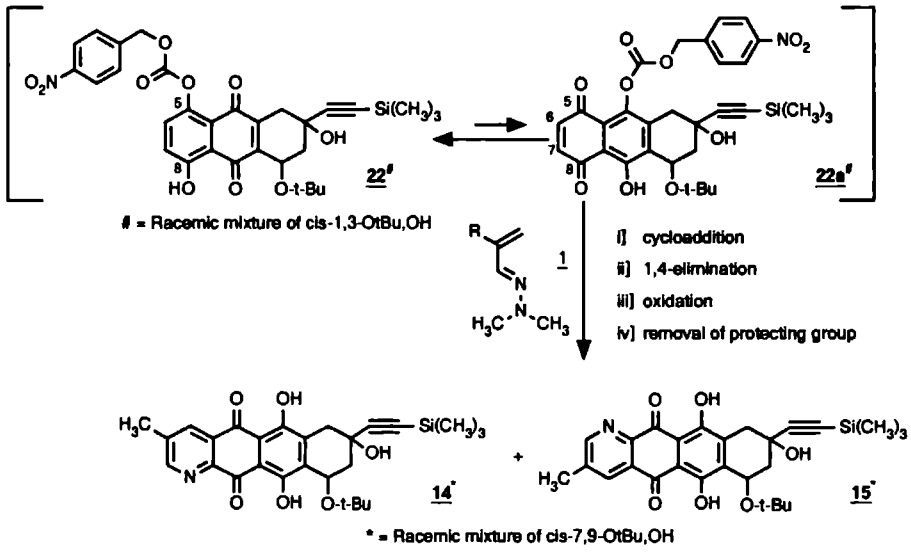


After reaction under high pressure conditions, a maximum of 25-30% of aromatized cycloadducts **14** and **15** were isolated. The regioselectivity of the Diels-Alder reaction depended on the dienophile used. A ratio of 1:2 between the formed products **14** and **15** was found. Separation of the mixture of **14** and **15** was possible using column chromatography, but was rather difficult because of absorption of nitrogen containing molecules to the column material. The Diels-Alder reactions of **13** with the diene **4** has also been performed at normal pressure giving the Diels-Alder adducts in the same ratios but lower isolated yields as described above. Subsequently, 1,4-elimination of the dimethylamine and oxidation either with $Pb(OAc)_4$ or air oxidation on silicagel gave the aromatic products **14** and **15**.

The azadiene reacts as a 1-substituted electron-rich diene having the highest HOMO coefficient at the γ atom. Analogously to the cycloadducts obtained from the reaction of **13** with 1 substituted electron-rich alkenes, compound **15** (see Scheme 6.1) is expected to be the major regioisomer (see also Chapter 3, compounds **9** and **10**). Further evidence was obtained from the NMR data of the regioisomers. The largest shift difference between both aromatic hydroxy protons was found for **14**. This was comparable with the shift difference in daunomycinone and its regioisomer[7]. NOE-difference NMR spectroscopy also gave the same indication, although very small, for the assignment of structures **14** and **15**. For compound **14**, a small NOE effect was found on protons at C_4 and C_7 of the A and D rings after irradiation of one of the aromatic hydroxy protons (see Scheme 6.2 for numbering). Attempts to crystallize one of these compounds for X-ray diffraction have not yet been successful.

In order to influence the regiochemistry and to make 14 more readily available and, also to find additional proof for the regiochemistry, dienophile 22 was used in the Diels-Alder reaction with diene 4 (Scheme 6.4)

Scheme 6.4



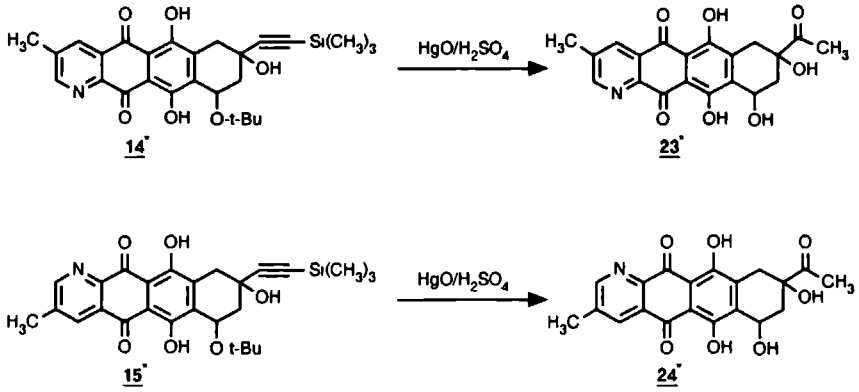
For compound 22, we expected the highest LUMO coefficient to be on carbon atom 6 (opposite to the hydrogen bridge, see also Chapter 2). In the cycloaddition with diene 4, this should lead to cycloadduct 14 (Scheme 6.4). Compound 22 was synthesized from 13 by regioselective protection of the 5-hydroxy group with CaH_2 and *p*-nitrobenzyl chloroformate (see also Chapter 3). In Chapter 2, we showed that the regioselective protection of related anthraquinones influenced the regioselectivity of the Diels-Alder reaction.

The ratio of 14 : 15 (1 : 2) from the reaction of diene 4 and dienophile 13 changed to 7 : 3 when dienophile 22 was used. The *p*-nitrocarbobenzoxy group was spontaneously removed under these basic reaction conditions. The moderate regioselectivity found in the conversion of 22 can be ascribed to partial removal of the protecting group from 22 before reaction with diene 4. This may be caused by dimethylamine liberated after the cycloaddition reaction.
The ratios were determined using ^{1}H-NMR spectroscopy from integration of the aromatic hydroxy protons between 13 and 14 ppm. As we needed both pyridine D ring analogs of daunomycinone, we applied both dienophiles 13 and 22 in combination with diene 4 for the preparation of the cycloadducts 14 and 15.

The 2-trimethylsilylethynyl functionality of compounds 14 and 15 was hydrolysed into the desired acetyl group by treatment with mercury (II) oxide and dilute sulfuric acid (Scheme 6.5). The hydrolysis of these compounds was much slower than for the non-pyridine D ring analogs.

The reaction was, however, accelerated by using an ultrasonic cleaning bath. We then observed subsequent partial deprotection of the *tert*-butoxy group. Compounds 23 and 24 were formed in an overall yield (for hydrolysis and deprotection) of 80-90%.

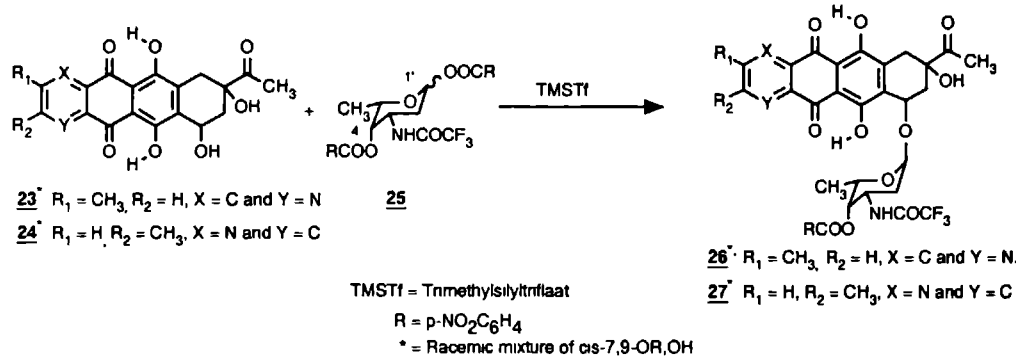
Scheme 6 5



6.3 Synthesis of D ring pyridine analogs of daunomycin.

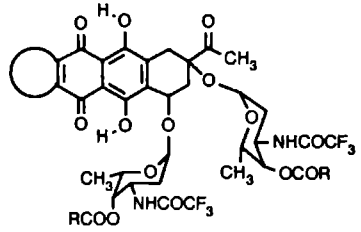
The racemic aglycones 23 and 24 were coupled with the appropriately protected L-daunosamine 25 under the conditions developed by Terashima and colleagues[9]. Successive addition of trimethylsilyltriflate and a solution of the aglycone in dichloromethane to a suspension of molecular sieves 4Å and sugar 25 in a mixture of anhydrous diethyl ether and anhydrous dichloromethane at -15°C for 3 h gave an inseparable mixture of the two diastereomeric α-glycosides 26 and 27 (Scheme 6.6). Only α-glycosides were formed due to stabilization of the intermediate cation at C_1' by the lone pair of the oxygen from the *p*-nitrobenzoyl group at C_4'[9].

Scheme 6 6



In this way glycosides **26** and **27** were isolated in a yield of 42% and 41%, respectively, as a mixture of diastereomers. In addition to small amounts of diglycoside **28**, the unreacted aglycone was recovered and could be used again.

Figure 6 4

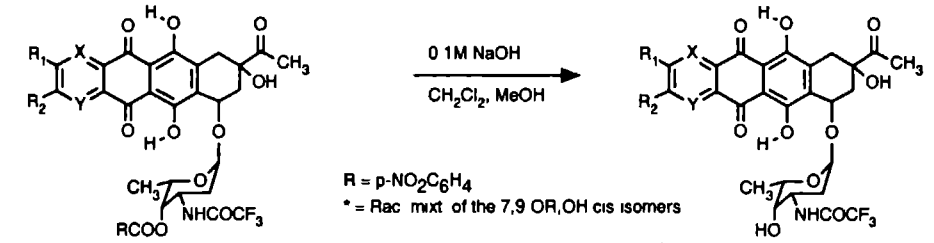


28 R = p NO_2 Ph-
Racemic mixture of cis-7,9-OR,OH

Chromatographic separation of this mixture of diastereomers was not possible using a variety of solvent combinations.

The N,O-diprotected α-glycosides **26** and **27** were deprotected (Scheme 6.7) at the 4'-hydroxy group by adding one equivalent of sodium hydroxide to a solution of the glyscoside in dichloromethane and methanol, to give the N-protected α-glycosides **29** and **30** in a yield of 40% - 50% (mixture of diastereomers).

Scheme 6 7



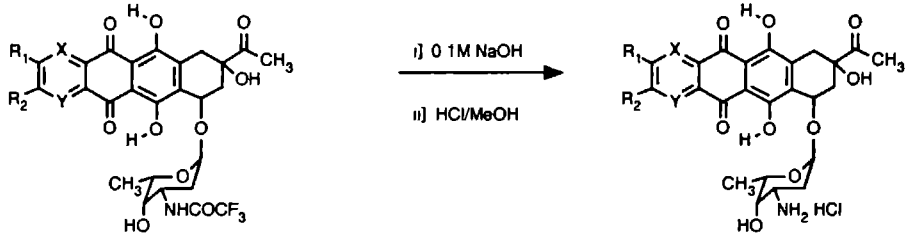
26* $R_1 = CH_3$, R_2 = H, X = C and Y = N
27* R_1 = H, $R_2 = CH_3$, X = N and Y = C

29* $R_1 = CH_3$, R_2 = H, X = C and Y = N
30* R_1 = H, $R_2 = CH_3$, X = N and Y = C

Formation of the biologically more active HCl salts of the pyridine D ring analogs of daunomycin **31** and **32** can be accomplished by deprotection of the trifluoroacetate in a solution of 0.1M sodium hydroxide followed by addition of 0.6M HCl in methanol (Scheme 6.8).

All HCl salts of the pyridine D ring derivatives have been tested in vitro for anti-tumor activity (see Chapter 8). Since all compounds were tested as mixtures of diastereomers and the diastereomeric mixture of **32** shows an acceptable activity, we tried to separate those diastereomers. In the ^{1}H-NMR spectra of **29** and **30** it was clearly seen that two diastereomers were present, in a ratio of approximately 1:1 (integration of the aromatic hydroxy protons).

Scheme 6.8



29* $R_1 = CH_3$, $R_2 = H$, X = C and Y = N

30* $R_1 = CH_3$, $R_2 = H$, X = N and Y = C

31* $R_1 = CH_3$, $R_2 = H$, X = C and Y = N.

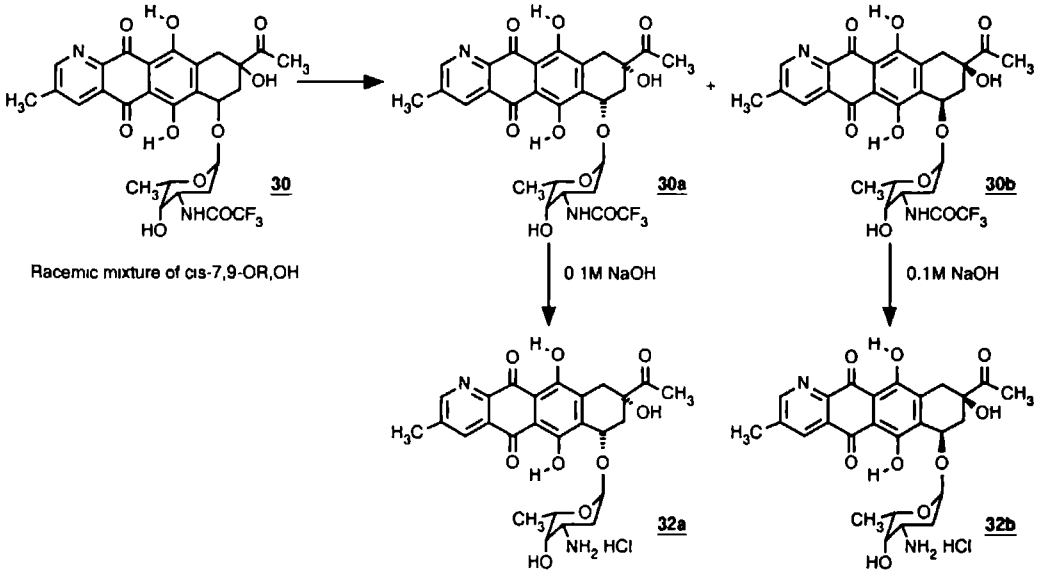
32* $R_1 = H$, $R_2 = CH_3$, X = N and Y = C

* = Racemic mixture of cis-7,9-OR,OH

The shifts of the aromatic hydroxy protons for the two diastereomers of compound 29 were clearly different (13.65 ppm and 13.81 ppm, respectively). For compound 30, these values were 13.43 ppm and 13.58 ppm.

Efforts to separate N,O-diprotected 27 were, as earlier mentioned, not successful. We did not try to separate the diastereomeric mixture of 32, because of its low solubility in organic solvents (32 is only soluble in water and in methanol) and its instability when using chromatography.
Therefore, our efforts were focused on the separation of the distereomers of 30. With centrifugal thin-layer chromatography using chloroform, methanol (2%) and glacial acetic acid (0.4%) as eluent, we were able to separate the diastereomers 30a and 30b (Scheme 6.9).
Both diastereomers were converted into their HCl salts (32a and 32b) and tested for anti-tumor activity (see Chapter 8).

Scheme 6 9



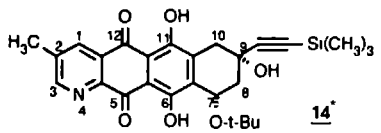
6.4 Conclusions

New pyridine D ring analogs of daunomycinone have been synthesized by an extension of the method as described in Chapter 3. Cycloaddition between dienophile 13 and diene 4 and subsequent hydrolysis resulted in the formation of two new daunomycinone analogs with a nitrogen atom at the 4 and 1 positions and a methyl group at 2 and 3 positions, 23 and 24, respectively. When using *p*-nitrobenzyloxycarbonyl-protected dienophile 22, the regioselectivity of the Diels-Alder reaction was reversed. The Diels-Alder reactions were performed under high pressure to reduce the reaction time and to increase the yields. Quite surprisingly, 1,4-elimination of dimethylamine also occurred under high pressure.

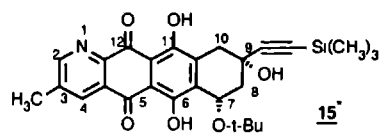
Both daunomycinone analogs 23 and 24 were coupled with the chiral protected sugar L-daunosamine according to the method developed by Terashima *et al.*[9]. After removal of the protecting groups both pyridine D ring analogs of daunomycin were isolated as a mixture of diastereomers 31 and 32. For analog 32, we were also successful in the separation of both diastereomers 32a and 32b.

6.5 Experimental section.

General remarks:
^{1}H-NMR-spectra were measured either on a Bruker WH-90 spectrometer or an Bruker AM-400 spectrometer using Me_4Si as internal standard. $CDCl_3$ was used as solvent unless otherwise stated. Melting points were taken using a Reichert Thermpan microscope and are uncorrected. Mass spectra were taken using a double focusing VG 7070 E mass spectrometer. For column chromatography, either Merck Silicagel 60 or Merck silicagel Art. 9385 (flash chromatography) were used. Chromatotron Model 7924T from Harrison Research (Palo Alto, US) was used for centrifugal thin-layer chromatography. Dry dichloromethane was distilled from CaH_2 and dry diethyl ether was distilled from sodium benzophenone ketyl. Obtaining correct elemental analysis for anthracyclines is very difficult. In several cases, one molecule of water was included in the molecule. Also in literature[13] examples have been published that one molecule of dichloromethane was included. Sometimes no analysis is given. Compounds must be carefully and repeatedly crystallized to obtain satisfactory analytical data. We tried to obtain satisfactory analyses for the synthesized compounds. Unfortunately not in all cases we have been successful. The numbering used for the compounds of this experimental section is illustrated below for structures 14 and 15. Both 14 and 15 are racemic mixtures of cis isomers.



14*



15*

* = Racemic mixture of cis-7,9-OtBu,OH

Cis-(+/-)-7,8,9,10-tetrahydro-4-aza-7-tert-butoxy-2-methyl-6,9,11-trihydroxy-9-(trimethylsilyl-ethynyl)-naphthacene-5,12-dione (14) and *Cis-(+/-)-7,8,9,10-tetrahydro-1- aza-7-tert-butoxy-3-methyl-6,9,11-trihydroxy-9-(trimethylsilylethynyl)-naphthacene-5,12-dione* (15).

0.75 g (6.6 mmol) of 2-methyl-2-propenal-N,N-dimethylhydrazone 4[11] was added to a solution of 1.07 g (2.5 mmol) of 13 in 15 ml of dichloromethane and divided over two high

pressure reaction vessels of 7.5 ml. A catalytic amount of hydroquinone was added to avoid polymerization and the reaction vessels were kept under high pressure (12 kbar) for 5 hours at room temperature. The reaction mixture was evaporated *in vacuo* and stirred for 1 h in 20 ml of diethyl ether to extract the excess diene and dienophile. The orange solid was filtered off and separated by column chromatography (1% methanol in dichloromethane) to give 100 mg (8%) of **14**, 270 mg (22%) of **15** and 420 mg (40%) of a mixture of **17/18**.

Compound **14**: m.p. 229-231°C; MS (FAB+) *m/z*: 494 (MH^+); EI peakmatch: found: 493.19207 (calc. 493.1920); ^{1}H-NMR (400 MHz): δ = 0.20 ppm (9H, s, $Si(CH_3)_3$), δ = 1.41 ppm (9H, s, $C(CH_3)_3$), δ = 2.01 ppm (1H, d, J=14.5 Hz, H_8(ax)), δ = 2.59 ppm (3H, s, 3-CH_3), δ = 2.76 ppm (1H, d, J=14.5 Hz, H_8(eq)), δ = 3.05 ppm (1H, d, J=20 Hz, H_{10}(ax)), δ = 3.67 ppm (1H, d, J=20 Hz, H_{10}(eq)), δ = 5.37 ppm (1H, brs, H_7), δ = 5.91 ppm (1H, s, 9-OH), δ = 8.39 ppm (1H, s, ArH), δ = 8.91 ppm (1H, s, ArH), δ = 13.23 ppm (1H, s, ArOH), δ = 13.80 ppm (1H, s, ArOH); Anal. calc. for $C_{27}H_{31}NO_6Si$: C, 65.70; H, 6.33; N, 2.84. Found: C, 65.07; H, 6.33; N, 2.78.

Compound **15**: m.p. 258-260°C; MS (FAB+) *m/z*: 494 (MH^+); ^{1}H-NMR (400 MHz): δ = 0.22 ppm (9H, s, $Si(CH_3)_3$), δ = 1.41 ppm (9H, s, $C(CH_3)_3$), δ = 2.01 ppm (1H, d, J=14.5 Hz, H_8(ax)), δ = 2.59 ppm (3H, s, 3-CH_3), δ = 2.76 ppm (1H, d, J=14.5 Hz, H_8(eq)), δ = 3.08 ppm (1H, d, J=20 Hz, H_{10}(ax)), δ = 3.70 ppm (1H, d, J=20 Hz, H_{10}(eq)), δ = 5.37 ppm (1H, brs, H_7), δ = 5.87 ppm (1H, s, 9-OH), δ = 8.44 ppm (1H, s, ArH), δ = 8.94 ppm (1H, s, ArH), δ = 13.46 ppm (1H, s, ArOH), δ = 13.56 ppm (1H, s, ArOH); Anal. calc. for $C_{27}H_{31}NO_6Si$: C, 65.70; H, 6.33; N, 2.84. Found: C, 65.31; H, 6.31; N, 2.87.

Compound **17/18**: ^{1}H-NMR (400 MHz, $CDCl_3$, CD_3OD): δ = 0.23 ppm (9H, s, $Si(CH_3)_3$), δ = 2.18-2.21 ppm (2H, H_8), δ = 2.70 ppm (3H, s, 3-CH_3), δ = 2.94-2.96 ppm (2H, H_7)), δ = 3.16 ppm (1H, d, J=18.5 Hz, H_{10}(ax)), δ = 3.31 ppm (1H, d, J=18.5 Hz, H_{10}(eq)), δ = 3.97 ppm (1H, brs, 9-OH), δ = 8.54 ppm (1H, s, ArH), δ = 8.97 ppm (1H, s, ArH), δ = 13.33 ppm (1H, s, ArOH), δ = 13.52 ppm (1H, s, ArOH); Anal. calc. for $C_{23}H_{23}NO_5Si$: C, 65.54; H, 5.50; N, 3.32. Found: C, 65.84; H, 5.58; N, 3.34.

*Diels-Alder reaction of diene **4** and dienophile **22**.*

0.75 g (6.6 mmol) of 2-methyl-2-propenal-N,N-dimethylhydrazone **4**[11] and 1.5 g (2.5 mmol) of **22**[12] in 15 ml of dichloromethane were converted as described for dienophile **10** to give 260 mg (22%) of **14** and 110 mg (9%) of **15**. The compounds were identical as in the previous reaction.

*Cis-(+/-)-9-acetyl-4-aza-2-methyl-6,7,9,11-tetrahydroxy-7,8,9,10-tetrahydronaphthacene-5,12-dione (**23**) and Cis-(+/-)-9-acetyl-1-aza-3-methyl-6,7,9,11-tetrahydroxy-7,8,9,10- tetrahydronaphthacene-5,12-dione (**24**).*

0.3 g (1.38 mmol) of mercury(II) oxide was added to a solution of 0.57 g (1.16 mmol) of **14** or **15** in 22 ml of dichloromethane and 11 ml of 6N sulfuric acid. The reaction mixture was sonicated in an ultrasonic cleaning bath for 24 h and then poured into 75 ml of 1M HCl. The reaction mixture was extracted with chloroform (3 x 50 ml) and the organic phase was dried over anhydrous sodium sulfate. After filtration, evaporation *in vacuo* and purification by column chromatography (chloroform/methanol/glacial acetic acid, 40 : 1 : 0.4) gave 0.38 g (86%) of **23** or **24**.

Compound **23**: m.p. 163-165°C; MS (EI+) *m/z*: 383 (M^+), peakmatch found: 383.10062 (calc. 383.1005); ^{1}H-NMR (400 MHz) : δ = 2.19 ppm (1H, dd, J=14.5 Hz and J=4.9 Hz, H_8(ax)), δ = 2.35 ppm (1H, dt, J=14.5 Hz, H_8(eq)), δ = 2.44 ppm (3H, s, $COCH_3$), δ = 2.60 ppm (3H, s, 3-CH_3), δ = 2.99 ppm (1H, dt, J=18.7 Hz and J=2.2 Hz, H_{10}(ax)), δ = 3.22 ppm (1H, dd, J=18.7 Hz and J=2.2 Hz, H_{10}(eq)), δ = 3.77 ppm (1H, d, J=5 Hz, 7-OH), δ = 4.54 ppm (1H, s, 9-OH), δ = 5.36 ppm (1H, brs, H_7), δ = 8.46 ppm (1H, d, J=2 Hz, ArH), δ = 8.96 ppm (1H, d, J=2 Hz, ArH), δ = 13.42 ppm (1H, s, ArOH), δ = 13.68 ppm (1H, s, ArOH).

Compound **24**: m.p. 197-199°C; MS (EI+) *m/e*: 383 (M^+); ^{1}H-NMR (400 MHz) : δ = 2.20 ppm (1H, dd, J=14.5 Hz and J=4.8 Hz, H_8(ax)), δ = 2.39 ppm (1H, dt, J=14.5 Hz and J=2.1 Hz, H_8(eq)), δ = 2.44 ppm (3H, s, $COCH_3$), δ = 2.60 ppm (3H, s, 3-CH_3), δ = 3.00 ppm (1H, d, J=18.7 Hz, H_{10}(ax)), δ = 3.24 ppm (1H, dd, J=18.7 Hz and J=2.1 Hz, H_{10}(eq)), δ = 3.84 ppm (1H, d, J=6.5 Hz, 7-OH), δ = 4.51 ppm (1H, s, 9-OH), δ = 5.34 ppm (1H, brs, H_7), δ = 8.47 ppm (1H, d, J=2 Hz, ArH), δ = 8.96 ppm (1H, d, J=2 Hz, ArH), δ = 13.41 ppm (1H, s, ArOH), δ = 13.43 ppm (1H, s, ArOH).

4-Aza-4-demethoxy-2-methyl-4'-O-p-nitrobenzoyl-3'-N-trifluoroacetyldaunomycin and 7,9-bis-epi-4-aza-4-demethoxy-2-methyl-4'-O-p-nitrobenzoyl-3'-N-trifluoroacetyldaunomycin (**26**).

Under an argon atmosphere, 0.27 ml of trimethylsilyl triflate (1.4 mmol) was added to a suspension of 360 mg of **25** (0.66 mmol) and 2 g molecular sieves 4Å in a mixture 27 ml of dry dichloromethane and 23 ml of dry diethyl ether at -30°C. The mixture was stirred at 0°C for 1 h and then cooled to -15°C. A solution of 200 mg (0.52 mmol) of **23** in 55 ml of dichloromethane was added and the mixture was then stirred at -15°C for 3 h. Progress of the reaction was followed by TLC (ethyl acetate/benzene, 1 : 4). The mixture was poured into a vigorously stirred solution of 350 ml of saturated aqueous $NaHCO_3$ and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with 100 ml of water and 100 ml of brine, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The residue was purified by preparative centrifugal thin layer chromatography (dichloromethane/acetone, 9 : 1) to give, after trituration with n-hexane, 165 mg (42%) of **26** (mixture of diastereomers): m.p. 179-180°C; MS (FAB) *m/z*: 758 (MH^+); ^{1}H-NMR (400 MHz): δ = 1.25-1.28 ppm (3H, 2 x d, J=6.3 Hz, 5'-CH_3), δ = 1.98-2.23 ppm (3H, m, $H_{2'}$(ax)), $H_{2'}$(eq) and H_8(ax)), δ = 2.33-2.53 ppm (1H, m, H_8(eq)), δ = 2.42 and 2.44 ppm (3H, 2 x s, $COCH_3$), δ = 2.60 and 2.62 ppm (3H, 2 x s, 3-CH_3), δ = 2.95-3.08 ppm (1H, 2 x d, J=19.3 Hz, H_{10}(ax)), δ = 3.24-3.34 ppm (1H, 2 x d, J=19.3 Hz, H_{10}(eq)), δ = 4.28 and 4.51 ppm (1H, 2 x s, 9-OH), δ = 4.40-4.70 ppm (2H, m, $H_{3'}$, $H_{5'}$), δ = 5.25-5.75 ppm (3H, m, $H_{1'}$, $H_{4'}$ and H_7), δ = 6.61-6.66 ppm (1H, 2 x d, J=7.5 Hz, NH), δ = 8.25-8.35 ppm (4H, m, ArH), δ = 8.43-8.47 ppm (1H, ArH), δ = 8.90-8.97 ppm (1H, ArH), δ = 13.08 and 13.11 ppm (1H, 2 x s, ArOH), δ = 13.66 and 13.82 ppm (1H, 2 x s, ArOH).

1-Aza-4-demethoxy-3-methyl-4'-O-p-nitrobenzoyl-3'-N-trifluoroacetyl-daunomycin and 7,9-bis-epi-1-aza-4-demethoxy-3-methyl-4'-O-p-nitrobezoyl-3'-N-trifluoroacetyl-daunomycin (**27**).

Under an argon atmosphere, 0.21 ml of trimethylsilyl triflate (1.11 mmol) was added to a suspension of 283 mg of **25** (0.52 mmol) and 3.5 g molecular sieves 4Å in 22 ml of dry dichloromethane and 18 ml of dry diethyl ether at -30°C. The reaction mixture was stirred at 0°C for 1 h and then cooled to -15°C. 155 mg (0.41 mmol) of a solution of **24** in 45 ml of dichloromethane were then added and the reaction mixture was stirred at -15°C for 3 h. Progress of the reaction was monitored by TLC (ethyl acetate/benzene, 1 : 4). The mixture was poured into a vigorously stirred solution of 250 ml of saturated aqueous $NaHCO_3$ and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with 100 ml of water and 100 ml of brine, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The residue was purified by preparative thin-layer chromatography (dichloromethane/acetone, 9 : 1) to give, after trituration with n-hexane, 126 mg (41%) of **27** (mixture of diastereomers): m.p. 192°C (dec.); MS (FAB) *m/z*: 758 (MH^+); ^{1}H-NMR (400 MHz): δ = 1.26-1.31 ppm (3H, 2 x d, J=6.6 Hz, 5'-CH_3), δ = 1.97-2.23 ppm (3H, m, $H_{2'}$(ax)), $H_{2'}$(eq) and H_8(ax)), δ = 2.36-2.53 ppm (1H, m, H_8(eq)), δ = 2.42-2.45 ppm (3H, 2 x s, $COCH_3$), δ = 2.61-2.62 ppm (3H, 2 x s, 3-CH_3), δ = 3.00-3.11 ppm (1H, 2 x d, J=19.2 Hz, H_{10}(ax)), δ = 3.29-3.38 ppm (1H, 2 x d, J=19.2 Hz, H_{10}(eq)), δ = 4.23 and 4.43 ppm (1H, 2 x s, 9-OH), δ = 4.42-4.71 ppm (2H, m, $H_{3'}$, $H_{5'}$), δ = 5.34-5.68 ppm (3H, m, $H_{1'}$, $H_{4'}$ and H_7), δ = 6.37-6.47 ppm (1H, 2 x d, J=7.5 Hz, NH), δ = 8.27-8.35 ppm (4H, m, ArH), δ = 8.46-8.48 ppm (1H, ArH), δ = 8.96-8.98 ppm (1H, ArH), δ = 13.38 ppm (1H, s, ArOH), δ = 13.47 and 13.61 ppm (1H, 2 x s, ArOH). Anal. calc. for $C_{35}H_{30}F_3N_3O_{13}$(1 H_2O): C, 54.15; H, 4.12; N, 5.42. Found: C, 54.18; H, 3.84; N, 5.37.

4-Aza-4-demethoxy-2-methyl-3'-N-trifluoroacetyldaunomycin and 7,9-bis-epi-4-aza-4-demethoxy-2-methyl-3'-N-trifluoroacetyldaunomycin (**29**).

300 mg (0.4 mmol) of **26** were dissolved in 3.9 ml of dichloromethane and 150 ml of methanol at 0°C under an argon atmosphere. 4.0 ml of 0.1M sodium hydroxide was added and the reaction mixture was stirred under the same conditions for 20 minutes. Progress of the reaction was monitored by TLC (dichloromethane/acetone, 9 : 1). Glacial acetic acid was added to the reaction mixture until the color of the solution became bright orange. 240 ml of ethyl acetate and 240 ml of brine were added to the reaction mixture and the organic layer was washed twice with 80 ml of brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*.

The residue was purified by preparative thin-layer chromatography (5% methanol in chloroform) to give 106 mg (44%) of **29** (mixture of diastereomers): m.p. 183-185°C; ^{1}H-NMR (400 MHz): δ = 1.30 and 1.32 ppm (3H, 2 x d, J=6.6 Hz, 5'-CH_3), δ = 1.85-2.20 ppm (4H, m, H_2·(ax)), H_2·(eq), H_8(ax) and 2'-OH), δ = 2.32-2.50 ppm (1H, 2 x brd, J=14.8 Hz, H_8(eq)), δ = 2.41 and 2.43 ppm (3H, 2 x s, $COCH_3$), δ = 2.60 and 2.61 ppm (3H, 2 x s, 3-CH_3), δ = 2.99-3.10 ppm (1H, 2 x d, J=19.2 Hz, H_{10}(ax)), δ = 3.27-3.35 ppm (1H, 2 x d, J=19.2 Hz, H_{10}(eq)), δ = 3.63-3.73 ppm (1H, H_4·), δ = 4.22-4.48 ppm (2H, H_3· and H_5·), δ = 4.33 and 4.51 ppm (1H, s, 9-OH), δ = 5.28-5.57 ppm (2H, H_1· and H_7), δ = 6.71-6.77 ppm (1H, J=7.5 Hz, NH), δ = 8.46-8.48 ppm (1H, d, ArH), δ = 8.96-8.98 ppm (1H, 2 x d, ArH), δ = 13.16 ppm (1H, s, ArOH), δ = 13.65 and 13.82 ppm (1H, 2 x s, ArOH). Anal. calc. for $C_{28}H_{27}F_3N_2O_{10}$: C, 55.27; H, 4.47; N 4.60. Found: C, 54.91; H, 4.29; N, 4.55

1-Aza-4-demethoxy-3-methyl-3'-N-trifluoroacetyldaunomycin (**30a**) *and 7,9-bis-epi-1-aza--4-demethoxy-3-methyl-3'-N-trifluoroacetyldaunomycin* (**30b**).

207 mg (0.27 mmol) of **27** in 16 ml of dichloromethane and 110 ml of methanol at 0°C under an argon atmosphere were converted with 2.7 ml of 0.1M sodium hydroxide and the reaction mixture was stirred under the same conditions for 20 minutes. Progress of the reaction was monitored by TLC (dichloromethane/acetone, 9 : 1). Glacial acetic acid was added to the reaction mixture until the color of the solution became bright orange. 150 ml of ethyl acetate and 150 ml of brine were added to the reaction mixture and the organic layer was washed twice with 80 ml of brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was purified by preparative thin-layer chromatography (5% methanol in chloroform) to give 75 mg (45%) of **30** (mixture of diastereomers): ^{1}H-NMR (400 MHz): δ = 1.28-1.32 ppm (3H, 2 x d, J=6.6 Hz, 5'-CH_3), δ = 1.83-2.23 ppm (4H, m, H_2·(ax)), H_2·(eq), H_8(ax) and 2'-OH), δ = 2.33-2.53 ppm (1H, m, H_8(eq)), δ = 2.42-2.45 ppm (3H, 2 x s, $COCH_3$), δ = 2.60-2.61 ppm (3H, 2 x s, 3-CH_3), δ = 3.00-3.11 ppm (1H, 2 x d, J=19.2 Hz, H_{10}(ax)), δ = 3.29-3.40 ppm (1H, 2 x d, J=19.2 Hz, H_{10}(eq)), δ = 3.63-3.73 ppm (1H, H_4·), δ = 4.20-4.54 ppm (3H, 9-OH, H_3· and H_5·), δ = 5.25-5.55 ppm (2H, m, H_1· and H_7), δ = 6.37-6.47 ppm (1H, J=8.5 Hz, NH), δ = 8.46-8.48 ppm (1H, ArH), δ = 8.96-8.98 ppm (1H, ArH), δ = 13.40 ppm (1H, s, ArOH), δ = 13.43 and 13.58 ppm (1H, 2 x s, ratio ~ 1 : 1, ArOH).

It was possible to separate the diastereomers of **30** by centrifugal thin layer chromatography (0.4% glacial acetic acid, 2% methanol, 97.6% chloroform) to give 68 mg (26%) of **30a** and 35 mg (13%) of **30b** from 325 mg of **27**.

Compound **30a**: m.p. 164-167°C; α^{20}_D = 252° (c = 0.04 in dioxane); MS (FAB) *m/z*: 609 (MH^+); ^{1}H-NMR (400 MHz) : δ = 1.31 ppm (3H, d, J=6.6 Hz, 5'-CH_3), δ = 1.87 ppm (1H, m, J=13.2 Hz and J=4.0 Hz, H_2·(ax)), δ = 2.03 ppm (1H, dt, J=13.2 Hz and J=5.2 Hz, H_2·(eq)), δ = 2.10 ppm (1H, s, 4'-OH), δ = 2.16 ppm (1H, dd, J=14.8 Hz and J=4 Hz, H_8(ax)), δ = 2.35 ppm (1H, dt, J=14.8 Hz, J=2 Hz and J=2 Hz, H_8(eq)), δ = 2.43 ppm (3H, s, $COCH_3$), δ = 2.60 ppm (3H, s, 3-CH_3), δ = 3.01 ppm (1H, d, J=19 Hz, H_{10}(ax)), δ = 3.31 ppm (1H, dd, J=19 Hz and J=2 Hz, H_{10}(eq)), δ = 3.70 ppm (1H, brs, H_4·), δ = 4.20-4.32 ppm (3H, m, H_3·, H_5· and 9-OH), δ = 5.25 ppm (1H, dd, J=4 Hz and J=2 Hz, H_7), δ = 5.49 ppm (1H, d, J=4.0 Hz, H_1·), δ = 6.75 ppm (1H, brd, J=8.6 Hz, NH), δ = 8.45 ppm (1H, d, J=1.8 Hz, ArH), δ = 8.95 ppm (1H, d, J=1.8 Hz, ArH), δ = 13.38 ppm (1H, s, ArOH), δ = 13.41 ppm (1H, s, ArOH); Anal. calc. for $C_{28}H_{27}F_3N_2O_{10}$.1 H_2O: C, 53.68; H, 4.67; N, 4.47. Found: C, 53.76; H, 4.40; N, 4.56.

Compound **30b**: m.p. 139-142°C; α^{20}_D = -248° (c = 0.05 in dioxane); MS (FAB) *m/z*: 609 (MH^+); ^{1}H-NMR (400 MHz) : δ = 1.29 ppm (3H, d, J=6.6 Hz, 5'-CH_3), δ = 1.86 ppm (1H, dd, J=13.5 Hz and J=5.1 Hz, H_2·(ax)), δ = 1.92-2.05 ppm (3H, m, H_2·(eq), 4'-OH and H_8(ax)), δ = 2.41 ppm (3H, s, $COCH_3$), δ = 2.47 ppm (1H, brd, J=14.8 Hz, H_8(eq)), δ = 2.61 ppm (3H, s, 3-CH_3), δ = 3.08 ppm (1H, d, J=19.4 Hz, H_{10}(ax)), δ = 3.34 ppm (1H, dd, J=19.4 Hz and J=1.2 Hz, H_{10}(eq)), δ = 3.64 ppm (1H, brs, H_4·), δ = 4.27-4.36 ppm (1H, m, H_3·), δ = 4.47 ppm (1H, q, J=6.6 Hz, H_5·), δ = 4.48 ppm (1H, s, 9-OH), δ = 5.36 ppm (1H, d, J=3.3 Hz, H_1·), δ = 5.54 ppm (1H, brs, H_7), δ = 6.78 ppm (1H, brd, J=8.6 Hz, NH), δ = 8.46 ppm (1H, d, J=1.4 Hz, ArH), δ = 8.96 ppm (1H, d, J=1.4 Hz, ArH), δ = 13.38 ppm (1H, s, ArOH), δ = 13.56 ppm (1H, s, ArOH); Anal. calc. for $C_{28}H_{27}F_3N_2O_{10}$.1 H_2O: C, 53.68; H, 4.67; N, 4.47. Found: C, 53.37; H, 4.43; N, 4.49.

4-Aza-4-demethoxy-2-methyldaunomycin and 7,9-bis-epi-4-aza-4-demethoxy-2-methyl-daunomycin (31).

The deep purple solution of 96 mg (0.16 mmol) of a mixture of **29** in 20 ml of 0.1M sodium hydroxide was stirred for 30 minutes at room temperature under an argon atmosphere. Progress of the reaction was monitored by TLC (water/glacial acetic acid/methanol/chloroform, 12 : 26 : 54 : 160). The pH of the solution was adjusted to 8 with a solution of 1M HCl and extracted several times with 75 ml of chloroform until the organic layer showed no orange color of the product. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The residue was dissolved in a minimal amount of a mixture of chloroform and methanol (9 : 1). After 0.3 ml of 0.6M HCl in methanol and 50 ml of diethyl ether were added, 60 mg (69%) of the HCl salt precipitated as a mixture of diastereomers of **31**. MS (FAB) *m/z*: 575 (M^+).

1-Aza-4-demethoxy-3-methyldaunomycin (**32a**) *and 7,9-bis-epi-1-aza-4-demethoxy-3-methyl-daunomycin* (**32b**).

The deep purple solution of 63 mg (0.10 mmol) of a mixture of **30** in 12 ml of 0.1M sodium hydroxide was stirred for 30 minutes at room temperature under an argon atmosphere. Progress of the reaction was monitored by TLC (water/glacial acetic acid/methanol/chloroform, 12 : 26 : 54 : 160). The pH of the solution was adjusted to 8 with 1M HCl and extracted several times with 50 ml of chloroform until the organic layer showed no orange color of the product. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The residue was dissolved in a minimal amount of a mixture of chloroform and methanol (9 : 1). After 0.2 ml of 0.6M HCl in methanol and 25 ml of diethyl ether were added, 40 mg (70%) of the HCl salt precipitated as a mixture of diastereomers.

Mixture of diastereomers **32**: α^{20}_D = -67°, (c = 0.075 in methanol); MS (FAB) *m/z*: 575 (M^+); Compound **32a**: α^{20}_D = 106°, (c = 0.05 in methanol); MS (FAB) *m/z*: 513 (MH^+-Cl^-); ^{1}H-NMR (400 MHz, D_2O) : δ = 1.24 ppm (3H, d, J=6.6 Hz, 5'-CH_3), δ = 1.86-2.0 ppm (3H, m, H_2·(ax), H_2·(eq) and H_8(ax)), δ = 2.25 ppm (1H, brs, H_8(eq)), δ = 2.41 ppm (3H, s, $COCH_3$), δ = 2.52 ppm (3H, s, 3-CH_3), δ = 2.86 ppm (1H, d, J=18 Hz, H_{10}(ax)), δ = 3.07 ppm (1H, d, J=18 Hz, H_{10}(eq)), δ = 3.60-3.66 ppm (1H, m, H_3·), δ = 3.76 ppm (1H, brs, H_4·), δ = 4.26 ppm (1H, q, J=6.6 Hz, H_5·), δ = 4.87 ppm (1H, brs, H_7), δ = 5.45 ppm (1H, d, H_1·), δ = 8.31 ppm (1H, s, ArH), δ = 8.77 ppm (1H, s, ArH).

Compound **32b**: m.p. 168-170°C; α^{20}_D = -156°, (c = 0.045 in methanol); MS (FAB) *m/z*: 513 (MH^+-Cl^-); ^{1}H-NMR (400 MHz, D_2O) : δ = 1.19 ppm (3H, d, J=6.6 Hz, 5'-CH_3), δ = 1.82-2.15 ppm (4H, m, H_2·(ax), H_2·(eq), H_8(ax) and H_8(eq)), δ = 2.43 ppm (3H, s, $COCH_3$), δ = 2.52 ppm (3H, s, 3-CH_3), δ = 2.82 ppm (1H, d, J=18 Hz, H_{10}(ax)), δ = 3.00 ppm (1H, d, J=18 Hz, H_{10}(eq)), δ = 3.60-3.66 ppm (1H, m, H_3·), δ = 3.73 ppm (1H, brs, H_4·), δ = 4.29 ppm (1H, q, J=6.6 Hz, H_5·), δ = 4.96 ppm (1H, brs, H_7), δ = 5.35 ppm (1H, d, H_1·), δ = 8.13 ppm (1H, s, ArH), δ = 8.74 ppm (1H, s, ArH).

6.6 References and Notes.

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7 The chemical shifts of the aromatic OH protons of daunomycin and 1-methoxy-4-demethoxydaunomycin give an indication for the found shift difference of the pyridine D ring derivatives of daunomycin[8]. Next to the evidence earlier mentioned, the shift differeneces between both aromatic OH protons were used to assign the structures for compounds **14** (δ=13.23 and δ=13.80 ppm) and **15** (δ=13.46 ppm and δ=13.56 ppm).

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10 Kita *et al.*[4] used this ratio and concentration for the glycosidation of their 11-deoxy-pyridine analog.

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CHAPTER 7

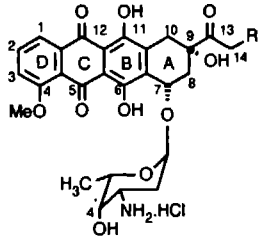
OVERVIEW OF BIOLOGICAL ACTIVITIES OF KNOWN DAUNOMYCIN AND ADRIAMYCIN DERIVATIVES.

7.1 Introduction.

The anthracyclines are the class of anti-tumor drugs compounds with the widest spectrum of activity against human cancers[1]. For more than 20 years daunomycin (1) and adriamycin (doxorubicin) (2, Figure 7.1) have been amongst the most widely prescribed and effective anti-tumor agent. Best results have been obtained against carcinoma of the ovary, lung, breast and leukemias.

The high therapeutic index of these anti-tumor drugs, and also the severe acute cardiotoxicity, has stimulated intensive research into the (semi-/total) synthesis of these compounds and their analogs. Most of these analogs have been obtained from semi-synthesis. Since isolation of daunomycin from a colony of *Streptomyces peucetius* was optimized and the compound was readily available, most chemists have used daunomycin as a starting material in their search for new, more active and less toxic derivatives[1a].

Figure 7.1



1 R = H

2 R = OH

Many groups have tried to accomplish total synthesis of daunomycin and its derivatives. Some of these approaches have been (partially) successful and several new derivatives have been synthesized and tested for their biological activity. However, so far, none of these new derivatives have been able to completely replace adriamycin or daunomycin in clinical use[1a].

In this chapter, an overview is given of which mechanisms of action are responsible for the anti-tumor activity of the anthracyclines. Their severe cardiotoxicity and attempts towards the development of new, less cardiotoxic derivatives are discussed. Examples of modifications of several moieties within the molecule are given and their influences on anti-tumor activity are summarized.

7.2 Anti-tumor activity and mechanism of action.

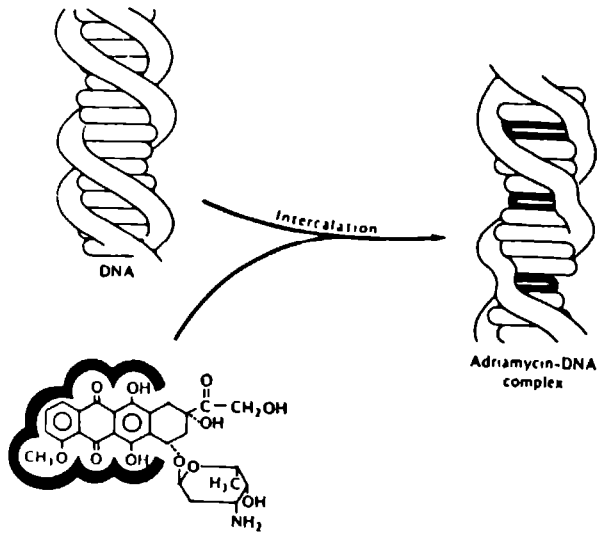
From reviews the following five mechanisms appear to have a major contribution to the anti-tumor activity of daunomycin and its derivatives:

1 Intercalation between DNA bases.
2 Free radical formation.
3 Covalent binding of reactive metabolites to DNA.
4 Topoisomerase II-mediated DNA strand breaks.
5 Interaction with the Cell membrane.

For the first four mechanisms, anthracycline must be present in the cell nucleus in order to become cytotoxic. In contradiction to this, the occurrence of cytotoxicity in the extracellular presence of adriamycin, linked to a polymer, supports evidence for an interaction of anthracyclines with the cell membrane.

Intercalation in the DNA strand (Figure 7.2) was the first mechanism proposed for the cytotoxic activity and is widely supported[2-5]. This intercalation will block DNA and RNA synthesis within the cell. Free radical formation (Figure 7.3), which might occur after intercalation, may result in DNA damage and would then contribute to cytotoxicity[6].

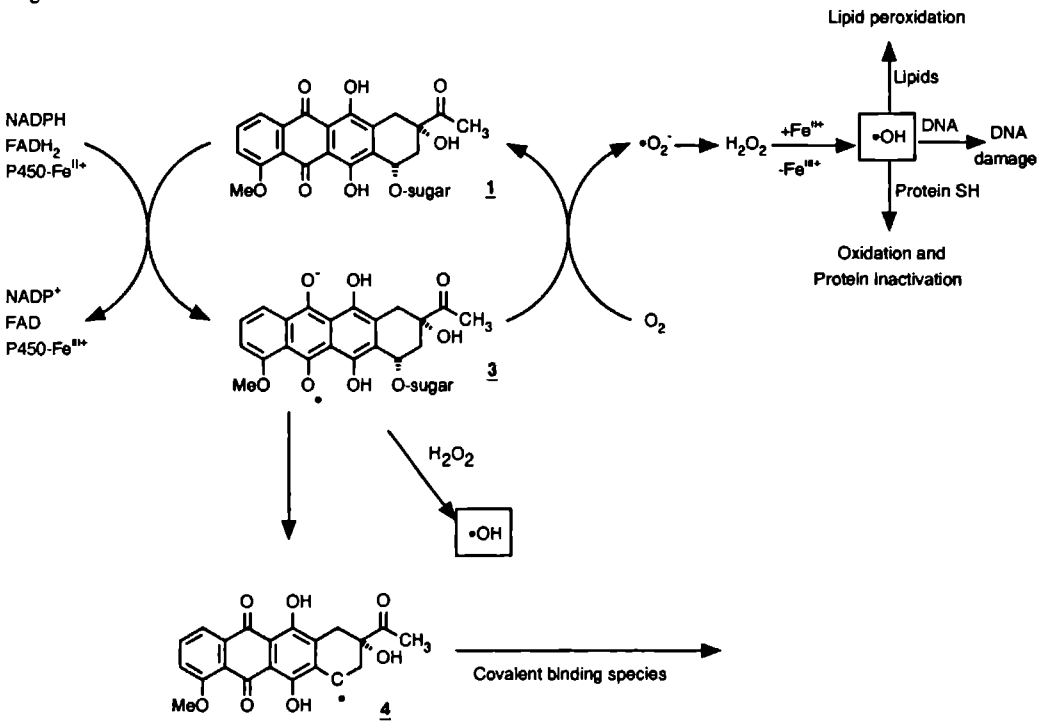
Figure 7.2



Daunomycin and other anthracyclines can undergo both one- and two-electron reduction to give the corresponding semiquinone (Figure 7.3, 3) or the dihydroquinone (Figure 7.5, 5)[7]. A range of flavin-centered, NADPH-dependent reductases are capable of accomplishing one-electron reduction of anthracyclines (1 → 3)[8]. The reduced drug can, in turn, reduce molecular oxygen to

superoxide ($\cdot O_2^-$) and/or hydrogen peroxide (H_2O_2)[9]. With hydrogen peroxide only, it can produce the highly reactive hydroxyl radical •OH. The formed superoxide is converted into hydrogen peroxide (Figure 7.3) which, in the presence of Fe^{II+}, is converted into the hydroxyl radical (•OH). It has been proposed that the anti-tumor activity of anthracyclines is due to the formation and reaction of •OH with cellular macromolecules (Figure 7.3)[9].

Figure 7.3



Furthermore, adriamycin[7] but not daunomycin, has been shown to be able to reduce Fe^{III+} to Fe^{II+}. Both the Fe^{II+} and the oxidized adriamycin is able to form the reactive •OH (Figure 7.4).

Figure 7.4

$$DX + Fe^{III+} \rightarrow DX\bullet \text{ (oxidized)} + Fe^{II+}$$

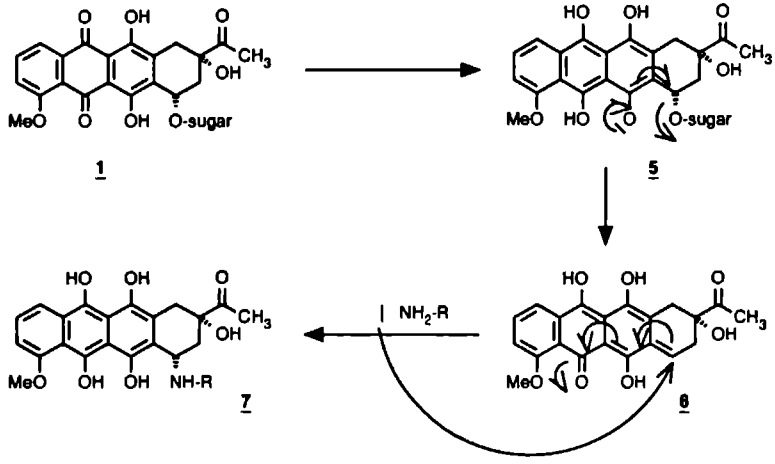
$$Fe^{II+} + H_2O_2 \rightarrow Fe^{III+} + \bullet OH + {}^-OH$$

$$DX\bullet + H_2O_2 \rightarrow DX + \bullet OH + {}^-OH$$

The hydroquinone 5 (Figure 7.5) formed directly via a two electron reduction can lose the sugar moiety to give the intermediate enone 6. This enone intermediate will react further with

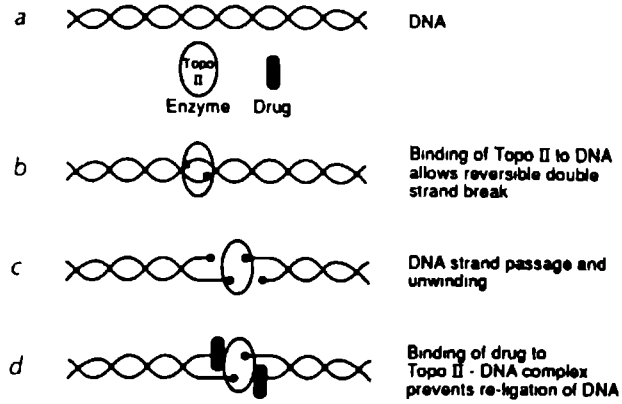
nucleophiles present in the cell (e.g., amines)[10].

Figure 7.5



Over the last few years, it has become clear that the capability of anthracyclines to stimulate the cleavage of DNA by the enzyme topoisomerase II (DNA forms a cleavable complex with topoisomerase II) must be considered as a potential mechanism of cytotoxicity (Figure 7.6[11]). This mechanism is also supported by the fact that adriamycin-resistant cell lines show a decrease in either the activity of topoisomerase II or in the formation of a cleavable complex with the drug[12a,b].

Figure 7.6



This resistance towards adriamycin treatment usually occurs after several treatment periods with the drug. The lack of response to one drug is usually accompanied by cross-resistance to several other, structurally unrelated agents. This is called Multi-Drug Resistance (MDR)[13], and it makes the development of new derivatives that lack cross-resistance or the co-administration of

so-called cross-resistance modifiers very important[14,14a].

The extracellular cytotoxicity of anthracyclines (mechanism 5) has been reviewed by Tritton[15]. In vitro experiments with adriamycin covalently linked with a polymer supported the theory that anthracyclines are also active without entering the cell[16]. The sequence starts with interaction of adriamycin on the cell membrane. This triggers a series of interactions that finally lead to DNA damage and death of the cell. Tritton also mentions unpublished data that intranuclear adriamycin alone cannot cause cytotoxicity. Extracellular adriamycin must also be present.

In conclusion, it can be stated that the mechanism of action of adriamycin and analogs is variable, complex and still only partially understood. For the cytotoxicity of anthracyclines, all of the discussed mechanisms will probably contribute to the cytoxicity and the overall effect.

7.3 Cardiotoxicity of anthracyclines.

Common side effects of most anticancer drugs are bone marrow toxicity and gastro-intestinal toxicity, manifesting as fatigue and nausea, respectively. The severity of the side effects determines the maximum tolerated dose (MTD). However, anthracycline treatment is more frequently accompanied by cardiotoxicity, which develops after repeated treatment. Congestive heart failure is often observed after treatment with high doses of anthracyclines or after prolonged treatment with lower doses[17a,b].

Biophysical studies have indicated that there is a strong electrostatic interaction between the positively charged amine of the anthracycline and highly anionic cardiolipins[18]. These cardiolipins, diphosphatidylglycerols, are a major component of mitochondrial membranes, which are present in abundance in cardiac tissue. A correlation between drug-cardiolipin association and cardiotoxicity was found. The formed complex is believed to alter the membrane environment and it can even cause cell death.
Free radical formation and consequent superoxide anion production, which are also responsible for the cytotoxicity, is also one of the causes for the severe cardiotoxicity[19]. The heart appears to be particularly sensitive to damage by free-radical attack[20]. Selective damage to the heart may also occur by formation of adriamycinol (the 13-dihydro metabolite formed by aldo-keto reductase from adriamycin), which seems to accumulate more than adriamycin in the heart. This and the fact that adriamycinol is equally active in the formation of free radicals makes it possible that the 13-dihydro metabolite is one of the major causes for this cardiotoxicity[21].

7.4 Approaches to more cytotoxic and less cardiotoxic new anthracycline derivatives.

In order to develop an anti-tumor drug with higher cytotoxic activity and substantially lower cardiotoxic effects many new derivatives of anthracyclines have been synthesized [1a,22,23].

Weiss[1a] estimated that the final number will be well in excess of 2,000. A large number have been evaluated at the National Cancer Institute, Bethesda, MD, USA. Most of these derivatives have been obtained by semi-synthesis; however this technique certainly limits the number of accessible derivatives.

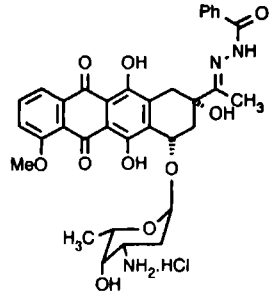
Within the molecules of daunomycin and adriamycin, there are 4 major moieties for modification (for numbering, see Figure 7.1):

1- Side chain modifications at C_{13} and C_{14}:

Functional sites which are easily accessible for semi-synthesis of new derivatives are C_{13} and C_{14} (Figure 7.1). The carbonyl at C_{13} has been reduced to the dihydro derivative or the 13-deoxy analog. The C_{13} carbonyl has also been functionalized by forming various hydrazones. Esters, ethers or amines of adriamycin have been obtained by reaction of 14-bromodaunomycin with sodium salts of carboxylic acids, alcohols or amines, respectively[22].

One of these derivatives, Zorubicin or Rubidazone 8 (Figure 7.7) is currently marketed in France and is active against acute leukaemias[1,22]. Several American investigators have, however, found that it has no advantages over daunomycin or adriamycin[1].
AD-32 9 (Figure 7.7), a derivative modified both in the side chain and sugar moiety, was discovered in 1973. At that time, drug formulation and solubility problems prevented further development after Phase I clinical trials, although the compound showed greater anti-tumor activity and less cardiotoxicity than the parent compound. Less toxicity in general was also found during preclinical testing. At the moment, this compound is being re-evaluated as a treatment for bladder cancer, and Phase I studies have been completed[1a].
Some of these derivatives look very promising in in vitro tests or even in in vivo tests, such as on L-1210 or P-388 cells (Figure 7.7) but, so far, none of them has been shown to be able to replace daunomycin or adriamycin in their clinical use[1a].

Figure 7.7



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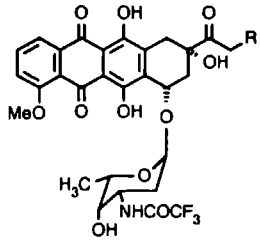


9: R = $OCO(CH_2)_3CH_3$

Test results P388 lymphotic leukemia system[24]

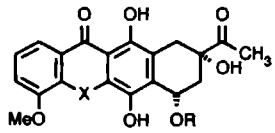
	A/P	T/C	O D.
1 daunomycin	0 79	179	1.00
2 doxorubicin	1 00	226	1.00
8 rubidazone	1.26	287	4.00
9 AD-32	1.34	303	30 00

2- Modifications in the BC rings:

With respect to redox potential and free radical formation, one can expect that modifications in the BC rings will have a great influence. Most of the modifications made to the BC rings of the anthracycline are obtained through total synthesis. Examples of this are the γ-pyrone (10) and γ-thiapyrone (11) derivatives. Compound 10 has been tested and appeared to be cytotoxic[25]. An influence on free radical formation and mechanistic considerations have been the motivation to synthesize these analogs. By modifying the quinone structure, chemists had hoped to influence both cytotoxicity and cardiotoxicity in a positive way.

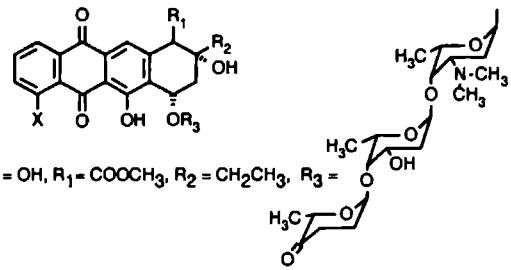
Another group of totally synthetic analogs were the deoxy analogs. Both the synthetic availability and the fact that aclarubicin (12, an active compound marketed in Japan and France) has no functionality at the 11 position led to chemists developing several 11-deoxydaunomycin derivatives (e.g., 13). An example of asymmetric synthesis of 11-deoxydaunomycinons has been published by Naruta *et al.*[26]. They also describe this type of derivative as more active and less toxic.

Figure 7 8



10 X = O, R = daunosaminyl

11 X = S, R = H

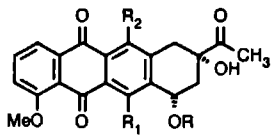


12 X = OH, R_1 = $COOCH_3$, R_2 = CH_2CH_3, R_3 =

13 X= OMe, R_1 = H, R_2 = $COCH_3$, R_3 = daunosaminyl

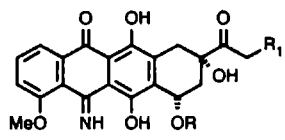
6- and 11-O-methyl derivatives (14 and 15) have also been developed to influence the redox potential of the quinone part. An unexpected BC ring imino derivative was found during treatment of daunomycin and adriamycin with methanolic ammonia. Although the 5-imino derivatives[27] 16 and 17 (more active and less cardiotoxic) looked very promising during in vivo and in vitro tests, they are not currently being investigated in Phase I or II clinical trials.

Figure 7 9



14 R_1 = OMe, R_2 = OH, R = daunosaminyl

15 R_1 = OH, R_2 = OMe, R = daunosaminyl



16 R_1 = H, R = daunosaminyl

17 R_1 = OH, R = daunosaminyl

None of the derivatives of daunomycin or adriamycin with a BC rings modification is currently being marketed or investigated in Phase I or II clinical trials[1].

3- Modifications in the D ring :

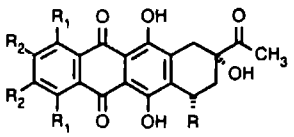
Modifications in the D ring are expected to have an influence on the redox potential and free radical formation.

One of the first D ring derivatives of daunomycin was carminomycin. This 4-demethyl derivative of daunomycin, also active against various tumors, was isolated by a Russian group[28] in 1973. The anti-tumor activity of this derivative appears to be inferior to that of adriamycin.

Another very promising derivative of daunomycin is 4-demethoxydaunomycin. This compound, synthesized by Arcamone and colleagues in 1976[29] was found to be much more active (T/C[24] of 261 versus 179 for daunomycin) and could be administered orally (both daunomycin and adriamycin must be intravenously injected). Although used worldwide for acute leukemias (intravenous only), 4-demethoxydaunomycin has not replaced daunomycin or adriamycin because of its limited bioavailability after oral administration.

Most of the other known D ring derivatives are only available via total synthesis. Only small amounts have been synthesized because of the often very long and difficult synthetic routes to the final products. Arcamone's group[30,31] have synthesized 1,4-dimethyl **18**, 2,3 dimethyl **19**, 1,4-dichloro **20**, 2,3-dichloro **21** and (2,3-a)benzo-4-demethoxydaunomycins **22**. All of these structures were tested in vitro for cytotoxicity and in vivo for anti-tumor activity in a L1210 leukemia in mice and the results are given in Figure 7.10. All analogs showed high cytotoxic activity, but in vivo tests showed lower anti-tumor activity compared to 4-demethoxy-daunomycin. The lack of efficacy of the chloro derivatives **20** and **21** was ascribed to a different in vivo metabolism.

Figure 7 10



R= daunosaminyl

Inhibition of colony-forming ability of cultuered HeLa cells after 24 hr exposure to drug, antitumor activity in L-1210 experimental leukemia in mice

	Structure	Cytotoxicity EC_{50}, ng/ml	Antitumor activity Dose, mg/kg	T/C (%)
daunomycin		10	2 9	144
4-demethoxydaunomycin		0 15	1 0	150
R_1=CH_3, R_2=H	**18**	10 05	6 6	147
R_1=H, R_2=CH_3	**19**	5 8	1 25	131
R_1=Cl, R_2=H	**20**	7 15	20	116
R_1=H, R_2=Cl	**21**	25	33 7	111
R_1=H, R_2=(2,3-a)dibenzo	**22**	27	10	135

More recently, the synthesis of 2-fluoro and 3-fluoro-4-demethoxydaunomycin have been published[32] as part of a program to probe the interaction of DNA with intercalating agents. No biological test results were given, but it would be interesting to compare the test results of these

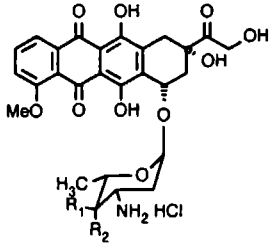
compounds with those of the chloro derivatives.

4- Modification in sugar moiety :

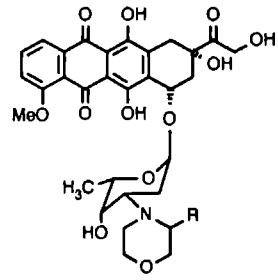
In the continuing search for new derivatives, many modifications have been made to the sugar moiety[33]. All natural sugars have been attached to the aglycone. Nowadays, new sugar derivatives are still being synthesized and tested[22].
One of the most successful and clinically used derivatives is 4'-epiadriamycin 23 (Figure 7.11). The difference with adriamycin is the equatorial hydroxy group at the 4 position of the sugar daunosamine. This derivative is active against different tumors[34] to daunomycin and adriamycin. The reduced cardiotoxicity of 4'-epiadriamycine (although the applied dose is higher) is also responsible for the fact that this compound is becoming used more often in the clinic[1]. In contrast to adriamycin, 4'-epiadriamycin is more rapidly metabolized and has the possibility to form glucuronides, which facilitates the excretion process[33a].

Other promising derivatives which are currently being evaluated in Phase I or Phase II clinical trials are 4'-deoxy-4'-iodoadriamycin 24[35] (Figure 7.11) and a series of morpholino adriamycins 25-27[36,37] (Figure 7.11). The morpholino compounds have a greater anti-tumor potency than adriamycin (25 was shown to be 40 times more potent than adriamycin in in vitro cytotoxicity assays), are less cardiotoxic and may have a different mechanism of action.

Figure 7 11



23 R_1=OH, R_2=H
24 R_1= H, R_2= I



25 R = H
26 R = CN
27 R = OMe

7.5 Conclusions.

Although more than 2,000 derivatives of daunomycin and adriamycin have been synthesized and some of them have been tested for biological activity, none has shown a clear cut advantage over adriamycin such that it has replaced the parent compound in the clinic. Many of the advantages observed in vitro (higher cytotoxicity and lower cardiotoxicity) disappeared in vivo or in Phase I clinical trials.

It seems that simple modifications of functional groups, such as esterfication of the hydroxy groups or iminisation of carbonyl groups, in the aglycone does not lead to better adriamycin derivatives. The question arises whether these simple modifications transform the new drug into a "prodrug". After administration, the parent is formed and, although the derivative looks very potent in in vitro or simple in vivo tests, no advantages in cytotoxicity or reduced cardiotoxicity are then to be found over the parent drug.

Changing the BC part of the aglycone has also not so far given better derivatives, although a tremendous influence was expected on the redox potential and the free radical formation.

The most promising derivatives for clinical use at the moment are idarubicin (4-demethoxy-daunomycin, only obtainable through total synthesis) and 4'-epirubicin (4'-epiadriamycin, **23**) having a modified sugar part. Together with daunomycin and adriamycin, these drugs are currently clinically used worldwide although 4'-epirubicin is not used in the USA. Three other anthracyclines are currently being marketed: in Japan and France: Pirarubicin (although 4'-tetrahydropyranyl derivative of adriamycin) and Aclarubicin **12**; and, in France only, Zorubicin **8**. The most promising new drugs in development (the morphonyl derivatives **25**-**27**) also have a modified sugar moiety.

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The P388 test results in Figure 7.7 are expressed as:
A/P = analog to parent ratio : T/C analog divided by the T/C of adriamycin.
T/C = median survival time of test animals times 100 divided by the median survival time of non-treated control animals.
O.D. = optimum dose, the dose which produces the highest T/C values in mg/kg/ injection.

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CHAPTER 8

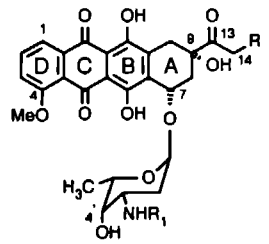
BIOLOGICAL ACTIVITIES OF NEW DERIVATIVES OF DAUNOMYCIN AND ADRIAMYCIN.

8.1 Introduction.

Although more than 2,000 derivatives of daunomycin (1, Figure 8.1) and adriamycin (2, Figure 8.1) have been synthesized[1] and their test results as anti-tumor agents have been disappointing[2], one has to realize that majority of the tested derivatives have been obtained by obvious transformations of functional groups in the aglycone. Relatively few compounds have been tested in which the aglycone was changed in such a way that can only be achieved by total synthesis.

Still less compounds have been tested in which both the aglycone and the sugar part are different from daunomycin or adriamycin, although AD-32 3 (Figure 8.1), a derivative with a C_{13},C_{14} side chain and sugar modification, shows the advantages of this approach[1].

Figure 8.1



1 R = H, R_1 = H.HCl

2 R = OH, R_1 = H.HCl

3 R = $OCO(CH_2)_3CH_3$, R_1 = $COCF_3$

In the preceding Chapters 5 and 6, total syntheses of derivatives of daunomycin have been described. The methods were originally developed for the total synthesis of daunomycin and 4-demethoxydaunomycin. However, the flexibility of our approach has allowed derivatization in several steps during the total synthesis.

In this chapter, we present the biological test results of some new derivatives which can only be obtained by total synthesis. Our main goal was to decrease the cardiotoxicity in comparison with the parent compound.

On the basis of the published results described in Chapter 7, we selected three possible moieties for modification which fit well in our synthetic approach:

- the acetyl side chain was replaced by an ethynyl group;
- the D ring was replaced by a pyridine ring;
- the amino group in the sugar part was protected by a trifluoroacetyl group.

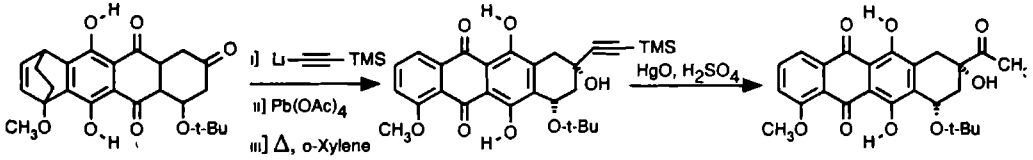
The flexibility of our total synthesis method allowed all possible combinations of these three alterations without major changes in the synthetic approach.

8.2 C_{13},C_{14} ethynyl analogs of daunomycin.

By replacing the acetyl group by the ethynyl group, we hoped, because another route of metabolism is expected, to prevent the formation of daunomycinol (13-dihydro daunomycin). As described earlier (section 7.3), the formation of daunomycinol may be one of the major contributions to cardiotoxicity, since the formed 13-dihydro analog tends to accumulate in the heart[3,4].

The synthetic route used provided the opportunity to test some new and promising side-chain derivatives of daunomycin. Although many groups have introduced the acetyl function by using ethynyllithium (for instance, Kelly *et al.*[5]), no one has reported the synthesis and testing of the ethynyl derivative of daunomycin. The introduction of the acetyl group at the 9 position of daunomycin was accomplished by addition of 2-trimethylsilylethynyllithium to **4** and hydrolysis of this ethynyl function **5** to the acetyl group **6** (Scheme 8.1).

Scheme 8.1



4* **5*** **6***

* = Racemic mixture of cis-7,9-OtBu,OH

Several ethynyl derivatives of daunomycin and 4-demethoxydaunomycin were synthesized and tested for biological activity (Figure 8.2, **7a** - **10a** and **7b** - **9b**). The synthesis of these compounds was described in Chapter 5.

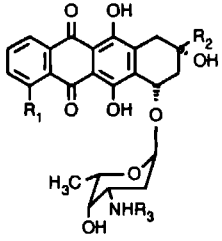
The compounds were tested by Dr. Lelieveld of the ITRI-TNO Institute, Rijswijk (The Netherlands) for in vitro cytotoxicity using a propidium iodide staining technique[6]. The method is similar to the MTT assay[7] with the exception that propidium iodide is used instead of a tetrazolium salt to quantify the number of cells.

The following 5 human tumor cell lines were used: A204 rhabdomyosarcoma (A cells), MCF-7 mammary carcinoma (M cells), T-24 bladder carcinoma (T cells), WiDr colon tumor (W cells) and IgR-37 melanoma (Z cells). These are representative for the cell types against which daunomycin and derivatives are usually active. As a reference, 4 known compounds (daunomycin **1**, adriamycin **2**, 4-demethoxydaunomycin **11a** and 7,9-bis-epi-4-demethoxy-daunomycin **11b**) were also tested at the same time in the same screens. In order to perform the experiment, the cell lines were maintained in continuous logarithmic cultures in Dulbecco's medium supplemented with 10% fetal bovine serum, together with penicillin (100 IU/ml) and streptomycin (100 μg/ml). The cells were mildly trypsinized for passage and for use in

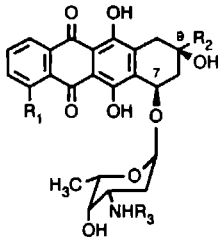
experiments. All compounds were dissolved in a small volume of DMSO and the solutions were diluted with full growth medium (Dulbecco's MEM + 10% fetal bovine serum) to the desired concentration. The plates with tumor cells were prepared and, after 2 days, different amounts of the test compounds were added. Each amount was tested in duplicate. After 5 days, the plates were treated with a special medium[7a] and cells were counted by a photomultiplier. This resulted in the IC_{50} values (the concentration at which 50% inhibition of cell growth occurs) as presented in Tables 8.1-8.3.

The results obtained from these test experiments for the compounds shown in Figure 8.2 are collected in Table 8.1. All compounds were tested blind under a coding system HRM.

Figure 8 2



7a R_1 = H, R_2 = C≡C-Si(Me)$_3$, R_3 = $COCF_3$ (HRM03)
8a R_1 = H, R_2 = C≡CH, R_3 = $COCF_3$ (HRM04)
9a R_1 = H, R_2 = C≡CH, R_3 = H HCl (HRM01)
10a R_1 = OMe, R_2 = C≡CH, R_3 = H HCl (HRM12)
1 R_1 = OMe, R_2 = $COCH_3$, R_3 = H HCl, daunomycin (HRM06)
2 HRM 08 R_1 = OMe, R_2 = $COCH_2OH$, R_3 = H HCl, adriamycin (HRM08)
11a HRM 05 R_1 = H, R_2 = $COCH_3$, R_3 = H HCl, 4-demethoxydaunomycin (HRM05)



7b R_1 = H, R_2 = C≡C Si(Me)$_3$, R_3 = $COCF_3$ (HRM07)
8b R_1 = H, R_2 = C≡CH, R_3 = $COCF_3$ (HRM02)
9b R_1 = H, R_2 = C≡CH, R_3 = H HCl (HRM09)
11b HRM 10 R_1 = H, R_2 = $COCH_3$, R_3 = H HCl, 7,9-bis-epi-4-demethoxydaunomycin (HRM10)

Table 8 1

IC_{50} VALUES OF ANTHRACYCLINES **1**, **2**, **7a** **10a**, **7b** **9b** and **11a**,**b** TESTED AGAINST HUMAN TUMOR CELL LINES

COMPOUND	A204	MCF 7	T24	WiDr	IgR37
1	1	1	4	3	1
2[7b]	5	5	16	12	5
11a	< 0 3	< 0 3	< 0 3	< 0 3	< 0 3
11b	243	420	728	372	469
7a	97	151	444	156	146
8a	14	14	75	43	18
9a	~0 3	1	2	4	2
10a	21	39	140	87	63
7b	2116	2846	6780	2495	16510
8b	1363	3212	4369	1047	3177
9b	302	745	1143	501	618

IC_{50} values in ng/ml (IC_{50}=the drug concentration at 50% inhibition of cell growth after 5 days of treatment)

In accordance with published results[8], our test results show that 4-demethoxydaunomycin **11a** is much more active than daunomycin **1** or adriamycin **2** in these test cell lines. These test results also confirm that the stereochemistry of the 7 and 9 positions is essential. The 7R,9R derivatives **7b**-**9b** and **11b** (optical isomers with unnatural configuration) are not active. Differences that can be identified in this series of analogs is the difference in lipophilicity. The IC_{50} values show a decrease in the sequence **7b** > **8b** > **9b** > **11b**, from which we may conclude that trifluoroacetyl protection of the nitrogen atom of the sugar moiety or trimethylsilyl protection of the ethynyl group deactivates the molecule.

By comparison of the 7S,9S analogs with the natural stereochemistry, the same conclusion about protection of the N atom or the ethynyl group can be drawn (**7a** > **8a** > **9a** > **11a**). The lowest IC_{50} values (below the detection limit of 0.3 ng/ml) were found for 4-demethoxydaunomycin. The values of the analog **8a** are quite low and almost comparable with those of adriamycin.

When we compared the IC_{50} values of the newly synthesized ethynyl derivatives **9a** (0.3-4) and **10a** (21-140) with the acetyl compounds **11a** (all < 0.3) and **1** (1-4) respectively, the conclusion is justified that replacement of the acetyl group by an ethynyl group deactivates the molecule with respect to cytotoxicity. It is interesting to note in this respect that the effects of D ring modification and C_{13},C_{14} side-chain modification are additive in these in vitro tests.

However, in comparison with the IC_{50} values of adriamycin, the IC_{50} values of both new analogs were sufficiently interesting to perform further testing (**9a**: slightly lower IC_{50} than adriamycin, values comparable with daunomycin and **10a**: values somewhat higher than adriamycin). If the cardiotoxicity of both compounds is considerably less than for daunomycin, these analogs might become interesting. For this reason, we finally selected 3 compounds (**8a**-**10a**) for further investigation in an in vivo test. The cardiotoxicity was also determined for these derivatives. The results are given in section 8.4, together with the results for the most promising D ring derivative.

8.3 Biological activity of new D ring analogs of daunomycin.

Only a few D ring derivatives have been synthesized and tested for anti-tumor activity because most of these derivatives can only be made by total synthesis. The most active, clinically used derivative of daunomycin is still the D ring derivative, 4-demethoxydaunomycin. Total synthesis of 4-demethoxydaunomycin has been the subject of many publications[9,9a]. The majority of these methods have been directed towards 4-demethoxydaunomycin[10] only and do not allow the synthesis of other D ring derivatives.

Results from the few derivatives tested show that alterations in the D ring have a strong effect

on cytotoxicity (see Chapter 7, section 7.2).

With the methods described in Chapters 2 and 3, it is possible to obtain new D ring analogs of daunomycin. The only limitation is the availability of precursor dienes.

8.3.1 Daunomycin analogs with a pyridine D ring.

The study of a daunomycin analog with a pyridine D ring was intriguing for several reasons. With respect to its shape, the molecule is closely related to the very potent 4-demethoxydaunomycin (**11a**). On the other hand, the pyridine ring will affect the redox potential of the quinone-BC part and, consequently, the formation of radicals (see paragraph 7.2). Finally, the nucleophilic nitrogen offers an additional position for protonation or interaction with electrophilic binding sites.

Our goal was to synthesize a non-substituted D ring with the N atom in either the 4 or 1 position. Because of the easy availability of substituted 1-aza-3-methylbutadienes and the published possibilities for Diels-Alder reactions with these dienes (see Chapter 6), we started with the synthesis of 4-aza-2-methyl-4-demethoxy (**16**) and 1-aza-3-methyl-4-demethoxy (**17**) analogs of daunomycin. The synthesis of these compounds is described in Chapter 6. Because of difficulties encountered with the separation of the 7S,9S and 7R,9R isomers, all of these compounds were first tested as mixtures of diastereomers. For analog **17**, we succeeded in the separation of both diastereomers (Figure 8.3, **17a**/**17b**) and these separated isomers were also tested for their cytotoxicity, together with **17**.
In view of the lower cytotoxicity found for the ethynyl analogs of 4-demethoxydaunomycin, we decided not to synthesize the corresponding ethynyl analogs of the pyridine D ring derivatives.

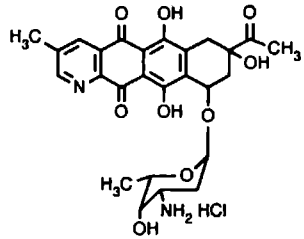
Table 8.2 shows the IC_{50} values of the hetereocyclic daunomycin derivatives. Again, as reference, the IC_{50} values for daunomycin **1**, adriamycin **2** and 4-demethoxydaunomycin **11a** are given. The same test method was used as described in section 8.2.
The position of the nitrogen atom in the D ring certainly seems to have an influence on the IC_{50} values. If compounds **16** and **17** are compared, a decrease in the IC_{50} values is observed when the nitrogen is in the 1 position.

Compound **17** was tested twice. In the first trial, when compounds **16** and **17** were tested, adriamycin was used as internal reference. In a second trial, when we tested compounds **17a** and **17b**, compound **17** was used as internal reference. Differences in IC_{50} values were found for all cell lines.

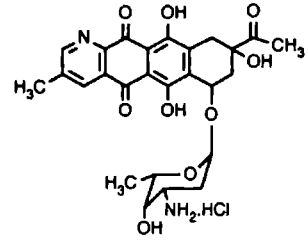
Although the differences are not very large, the conclusion is justified that compound **17** is the most active compound. In all five cell line tests, the lowest IC_{50} values were obtained for **17**.

Compound **16**, with the N atom at the 4 position and the methyl group at the 2 position, is clearly the least active compound.

Figure 8 3



16: (HRM15, Rac. mixt. of cis-7,9-OR,OH)



17: (HRM11/17, Rac mixt of cis-7,9-OR,OH)

Table 8 2

IC_{50} VALUES OF ANTHRACYCLINES **1**, **2**, **11a** and **14-17** TESTED AGAINST HUMAN TUMOR CELL LINES

COMPOUND	TRIAL	A204	MCF-7	T24	WiDr	lgR37
1	1	1	1	4	3	1
2	1	4	6	18	16	6
11a	1	< 0 3	<0 3	< 0 3	< 0 3	< 0 3
16	1	171	334	949	779	459
17*	1	45	67	173	139	77
17*	2	153	118	140	434	67
17a	2	15	17	17	38	7
17b	2	1378	1182	512	3625	713

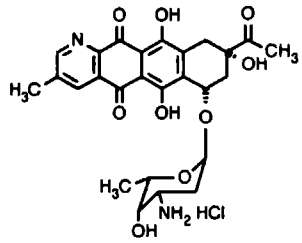
IC_{50} values in ng/ml (IC_{50}= the drug concentration at 50%inhibition cell growth after 5 days of treatment)
* compound **17** tested twice in different trials

Bearing in mind that the two compounds were tested as mixtures of diastereomers (7R,9R and 7S,9S) and the activities may increase using only the natural isomer, we decided to separate and test both isomers of compound **17** (Chapter 6). These test results are given in Table 8.3. For the determination of these IC_{50} values, compound **17** was also used as internal standard and, in Table 8.3, the adjusted values are given. Only this will allow us to compare the IC_{50} values of **17a** with those of daunomycin **1** and adriamycin **2**.

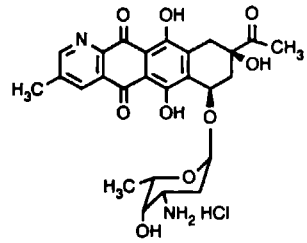
When we compare the IC_{50} values of adriamycin **2** with the adjusted IC_{50} values of compound **17a**, we see that these values are almost identical and that both compounds possess almost identical cytoxicity towards all tested cell lines.

Although there is no improvement in the cytotoxicity of compound **17a** compared with daunomycin **1** or adriamycin **2**, the decision was made to do additional tests with this compound. This decision was made because an acceptable cytotoxicity together with a reduced cardiotoxicity, and the possibility to overcome drug resistance, play an important role in the acceptance of a new anti-tumor drug in clinical use.

Table 8 3



17a (HRM 18)



17b (HRM 19)

IC_{50} VALUES OF ANTHRACYCLINES 17, 17a and 17b TESTED AGAINST HUMAN TUMOR CELL LINES
Values of 17a and 17b adjusted to internal standard compound 17

COMPOUND	A204	MCF 7	T24	WiDr	IgR37
2	4	6	18	16	6
17a	4	10	21	12	8
17	45	67	173	139	77
17b	405	671	632	1161	820

IC_{50} values in ng/ml (IC_{50} = the drug concentration at 50% inhibition of cell growth after 5 days of treatment)

Because of the difficulties encountered with the separation of 17a and 17b (see Chapter 6, section 6.2), compound 17 (a mixture of diastereomers) was used initially, prior to further testing. Together with the three compounds 8a-10a (see paragraph 8.2), compound 17 was tested in a L1210 in vivo system and the acute toxicity and cardiotoxicity were determined. The results of these test are described below.

8.4 In vivo test (L-1210) and (cardio)toxicity tests of new anthracyclines.

A: Acute toxicity: Maximum Tolerated Dose and/or 50% Lethal Dose (LD_{50}).

In order to establish the optimal dose for the L1210 in vivo tests, the acute toxicity of these drugs was estimated. For this purpose, mice were treated with a single intraperitoneal injection of the drug[11]. They were observed for a period of 30 days and acute drug toxicity was estimated by determining the MTD (Maximum Tolerable Dose) and/or the LD_{50} (Lethal Dose to 50% of test animals). These estimations (Table 8.4) were used in the determination of optimal doses for the L1210 in vivo test.

As can be expected from earlier published results[8], the tolerated doses of the N-trifluoroacetyl protected compound 8a are much higher than for the corresponding HCl salt 9a. The difference

between the ethynyl daunomycin derivative **10a** and the ethynyl 4-demethoxydaunomycin analog **9a** is also not unexpected. The value for the LD_{50} for **17** looks promising, certainly since the optimal doses of this compound are lower. The normally used optimal dose[13] for in vivo L1210 testing for daunomycin **1**, adriamycin **2** and 4-demethoxydaunomycin **11a** are 2.9, 2.9 and 0.6 mg/kg, respectively.

Table 8.4

LD_{50} values of compounds **8a** - **10a** and **17** as estimated by Lelieveld[13]

COMPOUND	Estimated LD_{50} value
8a	50-150 mg/kg
9a	8 mg/kg
10a	20-50 mg/kg
17	20-50 mg/kg

B: In vivo L1210 test. Mouse bearing lymphoblastic leukaemia.

The L1210 system (lymphoblastic leukaemia) is, together with the P388 system (lymphatic leukaemia system), one of the most used test systems to compare in vivo anti-tumor activity of new and existing drugs. After the in vitro test on human tumor cell lines, the most promising new analogs are usually tested in vivo to see whether the observed anti-tumor activity is still seen. If both tests are successful, the next step is usually a study against solid mouse tumors or against human tumors in nude mice. The leukaemia cell line L1210 arose in 1948 in the spleen and lymph nodes of an 8 month-old female DBA mouse following skin painting with 0.2% 3-methylcholanthrene in ether[12]. Since then, this lymphoblastic leukaemia in DBA/2 or related hybrid mice has been used for the determination of anti-tumor activity of drugs. The Radiobiological Institute of TNO in Rijswijk (The Netherlands), at which our compounds have been tested, introduced the L1210 leukaemia in 1969. The used method is described in reference 13.

The test results are given in Table 8.5. Compound **9a**, which looked very promising in the in vitro tests, now appears to be inactive and the T/C value is even less than 100%. As expected, the derivative of 4-demethoxydaunomycin was used at the lowest dose level. It is possible that the results would be better if the dose was lowered even further. At the tested dose levels the compound might still be too toxic and cause acute toxicity. All we see at the moment is an increase of the T/C value at lower doses. Further investigations will be necessary to establish the optimal dose level.

The N-trifluoroacetyl derivative **8a** was administered, as expected, at the highest dose level in this test. Reduction from 140 mg/kg to 70 mg/kg gave an increase in the T/C value (110 to 170).

A value of 170 is comparable with the value of adriamycin in other L1210 tests. The high dose level and the limited solubility (see also cardiotoxicity test) make the usability of this compound questionable. The T/C value might be further increased by lowering the dose level, as an optimal value between anti-tumor activity and (acute) toxicity is usually observed at a certain dose.

The daunomycin analog **10a** was tested in a separate trial. A group of 5 mice was tested and the variation in the dose was between 15 and 25 mg/kg. The variation in T/C values was between 140 and 180%. The fact that the dose of 20 mg/kg had the lowest T/C value was of some interest. As with **8a**, this compound was classified as active but not spectacularly so. The higher dose compared to the 4-demethoxy analog **9a** corresponds with the difference in dose/activity relationship between daunomycin and 4-demethoxydaunomycin.

Compound **17**, tested as a mixture of diastereomers, appeared to be very active at the tested level of 40 mg/kg (T/C = 225%). Increase or decrease of the dose was followed by a decrease in the T/C value. In this case, the optimum between anti-tumor activity and toxicity level was established. The fact that long-term survivors were found was also very promising. None of the other tested compounds resulted in any long term survivors. For this compound, the cardiotoxicity test became very important.

Table 8 5

In vivo anti-tumor activity of compounds **8a** - **10a** and **17** tested with DBA/2 mice bearing L1210 (lymphoblastic leukaemia)

COMPOUND	DOSE (mg/kg)	MEAN BODY WEIGHT (g)				MST (day)	T/C (%)	LTS
		day 1	day5	day 7	day 8			
control	0	25 1	25 9	27 4		10	100	0/9
8a	70	26 1	25 6	26 1		17	170	0/6
	100	26 0	23 5	24 3		13	130	0/6
	140	25 0	22 8	21 9		11	110	0/6
9a	4 5	25 0	22 6	21 6		9	90	0/6
	6 0	25 5	20 8	18 6		8	80	0/6
	8 0	25 6	19 9	18 1		7	70	0/6
10a	15	28			29	18	180	0/5
	20	29			29	14	140	0/5
	25	30			29	18	180	0/5
17	30	24 4	23 6	24 6		15	150	1/6
	40	25 9	23 6	24 8		22 5	225	2/6
	50	25 1	23 2	23 2		16	160	0/6

C: In vitro/in vivo test for cardiotoxicity.

Usually, the cardiotoxicity of anthracyclines and other anti-tumor antibiotics is determined by treatment of a group of animals with test compounds for a long period of time. Cardiac lesions

are then scored after death. Over the last few years, acute models have been developed to allow prediction of chronic cardiotoxicity[15]. An acute model is, of course, much faster than a chronic model and there are indications that chronic cardiotoxicity might be caused by accumulation of several acute effects[15]. These tests were performed at the Department of Oncology in cooperation with the Department of Pharmacochemistry of the Free University of Amsterdam (The Netherlands). The compounds were tested for their negative inotropic effect on the isolated mouse left atrium, as described by De Jong *et al.*[15].At several concentrations of the drug, during a period of 2 hours, the decrease in the basal contractile force of the atrium was measured. The influence of the drug on the heart rate was established. A semi-logarithmic plot of the obtained ratios vs. the concentration was made (Dose Response Curve, DRC) and the IC_{50} (drug concentration resulting in 50% decrease of the initial (basal) contractile force) were determined. In addition to the newly synthesized compounds **8a** - **10a** and **17** adriamycin was measured as reference.

The results shown in Table 8.6 lead to the following conclusions.

- the solubility of **8a** is very low. The value measured at 30 μM is 78 ± 9%. Because micelles were even formed at 1 μM, it might be concluded that the free concentration in the organ bath was 1 μM or less. Adriamycin **2** gave a contractile force of 75 ± 8% at a concentration of 3 μM, which makes **8a** at least as cardiotoxic as adriamycin.
- compound **9a** is 4 times more cardiotoxic than adriamycin **2**.
- the cardiotoxicity level of **10a** is comparable with that of adriamycin (17.2 *vs* 21.9).
- compound **17** is less cardiotoxic than adriamycin. The values for these two compounds are 42.9 and 21.9, respectively.

Table 8.6

Cardiotoxicity test of compounds **2**, **8a** - **10a** and **17**

COMPOUND	Conc (μM)	Number of experiments	% of basal contraction force	IC_{50} (μM)	Correlation coefficient
2	3	2	75 ± 8	21.9	1.000
	10	4	60 ± 6		
	30	5	46 ± 7		
8a	no values measured because of limited solubility				
9a	3	4	60 ± 6	4.77	-
	10	3	32 ± 8		
	30	4	33 ± 2		
10a	3	2	84 ± 6	17.2	1.000
	10	4	61 ± 6		
	30	4	39 ± 7		
17	30	4	59 ± 6	42.9	0.985
	50	4	49 ± 16		
	100	3	22 ± 5		

8.5 Conclusions.

From the daunomycin and adriamycin analogs that we synthesized (Chapters 5 and 6), 15 were tested in vitro against five human tumor cell lines. The compounds with the unnatural stereochemical configuration (R,R) on the 7 and 9 positions (**7b** - **9b**) were expected to have low or no cytotoxicity. This was indeed the case, and no further development or testing has been carried out with these analogs. The other derivatives (**7a** - **10a**) with the natural stereochemical conformation (S,S) appeared to be active in these in vitro tests. Because of the relatively high in vitro activity compared to daunomycin and adriamycin, compounds **8a**, **9a** and **10a** were selected for further testing.

Compound **8a** is the N-trifluoroacetyl derivative of **9a**. As might be expected, when comparing for instance optimal doses in P388 tests of daunomycin and N-trifluoroacetyl-daunomycin[8], the LD_{50} value of the N-protected derivative was higher. However, when we compare the T/C values of **8a** and **9a**, the in vivo test showed a higher T/C value for **8a**. As explained earlier, the T/C value of **9a** may be higher at lower doses. The T/C value of **8a** also does not seem to be optimized. Further testing will be necessary to confirm these expectations, although cardiotoxicity tests show that both compounds are more cardiotoxic than adriamycin.

The in vivo test results for **10a** are rather confusing. The highest T/C values were found at 2 different dose levels (15 and 25 mg/kg), whereas at the dose level of 20 mg/kg the T/C is lower. When T/C values of daunomycin (T/C = 150 at 4 mg/kg[8]) and adriamycin (T/C = 141 at 2.9 mg/kg[8]) are compared with the value of **10a** (T/C = 180 at 15 mg/kg), it cannot be concluded that replacement of an acetyl of hydroxyacetyl group by an ethynyl group has a positive influence on the T/C. Although the T/C value is higher, the dose is also 4-5 times higher than for daunomycin and adriamycin. Certainly, **10a** is slightly more cardiotoxic than adriamycin. For **10a**, further tests at dose levels below 15 mg/kg are also required before the conclusion can be drawn that replacement of the acetyl group by the ethynyl group has a positive influence (or not) on the anti-tumor activity of the analog. A lower cardiotoxicity level would support the assumption that the metabolism routes of the tested compounds are different. Our results, however, do not allow such a conclusion at this moment.

The last group of tested analogs, obtained by total synthesis, contain a nitrogen atom in the D ring. Because of the difficulties that were encountered with the separation of the diastereomers (7R,9R and 7S,9S, configurations of the aglycones), all these compounds were tested as a mixture of diastereomers, which were present in almost equal amounts. This means that only 50% of the compound possess anti-tumor activity (compare analogs **11a** and **11b**, see Table 8.1). From the tested analogs (**16** and **17**), compound **17** appeared to have the highest in vitro activity. Although the cytotoxicity of **16** was only slightly lower the decision was made to do further testing only with **17**. Small amounts of both diastereomers were isolated and also tested in vitro.

Surprisingly, the natural isomer (7S,9S) appeared in average more than 8 times as potent as the mixture of diastereomers and was comparable with that of adriamycin (Table 8.2).

The mixture of diastereomers (17) was tested in vivo (L1210) for anti-tumor activity. After an estimation of the LD_{50} value (20-50 mg/kg), a T/C value of 225 (dose 40 mg/kg) was found for 17. Two long term survivors (> 60 days) were also reported. This means that those mice had recovered from the effects of the injection of tumor cells. Furthermore, the cardiotoxicity test showed that this analog was less cardiotoxic than adriamycin. These results are very promising and further testing is required. The next step will be a study against solid mouse tumors or against human tumors in nude mice. Before the next step is taken, more material must be made available. The synthesis of 17 must be optimized and the separation of diastereomers must be improved. In further testing, it is advisable to use only diastereomer **17a**. Cardiotoxicity then might be considerably lower and the L1210 test might results in even more long term survivors when repeated with **17a**.

8.6 References and Notes.

1 Lown, J.W., *Chemical Society Reviews*, **1993**, 165.

2 Weiss, R.B., *Seminar in Oncology*, 1992, *19(6)*, 670.

3 Olson R.D. and Mushlin P.S., *FASEB J.* **1990**, *4*, 3076.

4 In steroid chemistry, the replacement of an acetyl group by an ethynyl moiety has also led to very active derivatives of which the metabolism was different from the parent. The fact that an often used anti-contraceptive developed by Organon contained a steroid, ethynylestradiol, in which the acetyl group was replaced by an acetylene functionality, contributed to this idea. According to Zeele (Organon, personal communication), metabolism of this steroid is completely different from the original estradiol. The ethynyl group is not metabolized to the acetyl group. Nevertheless, this steroid is still active and the Organon product is one of the most sold anti-contraceptives.

5 Kelly, T.R., Ananthasubramanian, L., Borah, K., Gillard, J.W., Goerner, R.N., King, P.F., Lyding, J.M., Tsang, W.G. and Vaya, J., *Tetrahedron*, **1984**, *40(22)*, 4569.

6 Van Lambalgen, R. and Lelieveld, P., *Invest. New Drugs*, **1987**, *5*, 161.

7 Alley, M.C, Scudiero, D.A., Monks, A, *et al.*, *Cancer Research* **1988**, *48*, 589.

7a The special medium was developped by Van Lambalgen, R. and Lelieveld, P.[6] and contained Saline (100 µl), 0.002%; w/v propidium iodide (Sigma), 0.3% drawing ink (Staedler marsmatic 745) and 0.5% Triton X-100.

7b The IC_{50} values for adriamycin 2 which has been tested in two different trials do not differ much from each other or from previous determinations published by Lelieveld *et al.*[6]. In Tables 8.1-8.3, the values are given as an average of both trials.

8 Arcamone, F., *The Development of New Anthracycline Antibiotics, Anticancer Agents Based on Natural Product Models* (Ed. Cassady, J.M. and Douros, J.D.), Vol 16, Academic Press, New York, **1980**, 32.

9 Arcamone F., *'Doxorubicin'*, Medicinal Chemistry, Academic press, **1981**, *Vol. 17.*

9a Krohn, K., *Angew. Chem.*, **1986**, *98*, 788.

10 Gupta, R.C., Harland, P. and Stoodley, R.J., *J.Chem. Soc. Chem. Comm.*, **1983**, 754.

11 The MTD/LD50 test were performed by Dr. Peter Lelieveld from ITRI-TNO, Rijswijk. The test compound was suspended in a solution of 2% carboxymethylcellulose in saline. The suspension was administered intraperitoneally in a volume of 10 ml/kg body weight. The experiment was performed with 9-15 week old DBA/2 mice. Groups of two mice were treated with a single injection of 8, 20, 50 (and in one case 150) mg/kg body weight of the test compound. Body weights were determined immediately before the test and on

several occasions thereafter. The mice were observed for a period of 30 days. Signs of illness and mortality were recorded. At the end, the Maximum Tolerated Dose (MTD) and or the dose which is lethal to 50% of the mice (LD50) was estimated.

12 Law *et al.*, *J. Natl. Cancer Inst.*, **1949**, *10*, 179.

13 The L1210 leukaemia is a lymphoblastic leukaemia, transplantable in DBA/2 or related hybrid mice. After transplantation, there is a lag phase of 1-2 days, which is associated with implantation into the host. This period is followed by lop phase proliferation of the cells with a population doubling time of 10-14 hours[14]. When the leukaemic cells have grown to 1.5 x 10^9, they will cause death of the mice. The cell line is maintained by weekly transfer of 10^5 leukaemic cells injected i.p. into hybrid mice. Since 1990, male DBA/2 mice have been used as recipients in experimental work. On several occasions, batches of L1210 leukaemia were frozen in liquid nitrogen. Since 1980, an ampoule with cells is thawed at least twice a year. This procedure is followed to minimize the chance of changes in behaviour and/or properties of the leukaemic cells. Test method: At day 0, 10^5 L1210 cells were injected into a male DBA/2 mice. After 24 hours, the drugs were administered to a group of 6 mice. All drugs were suspended in 2% carboxymethyl-cellulose and administered intraperitoneally as a single injection in a volume of 10 ml/kg. Each drug was tested at three dose levels. The highest dose (approx. LD50 value) was expected to show some toxicity. The untreated control group consisted of 9 mice. The average body weight of the group was determined several times and the survival time of the mice was recorded. The results are expressed as median survival time (MST) of treated over control mice (T/C%). The number of long term survivors was also determined (> 60 days).

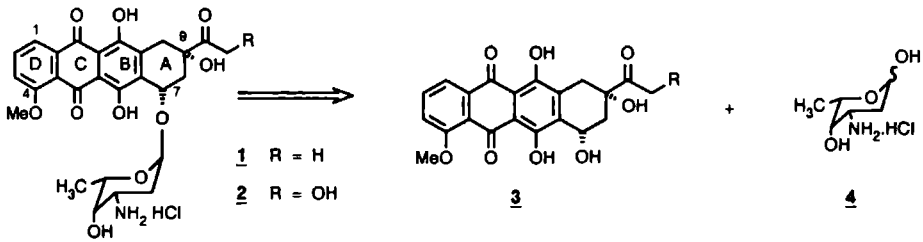
14 Skipper, *et al.*, *Cancer Chemother. Rep.*, **1964**, *35*, 1.

15 De Jong, J., Schoofs, P.R., Onderwater, R.C.A., Pinedo, H.M., Van der Vijgh,, W.J.F. and Bast, A., *Res. Commun. Chem. Pathol. Pharmacol.*, **1990**, *68*, 275.

SUMMARY

In 1957 a red pigment was isolated from a colony of *Streptomyces peucetius* which was called daunomycin (1, Figure S.1). Six years later the compound was shown to have an anti-tumor activity. In 1969 the isolation of adriamycin 2 from a variant strain of *Streptomyces peucetius* named caesius was reported. This compound, which is in clinical use since 1974, now still belongs to the most widely used anti-tumor antibiotics as it has the widest spectrum of anti-tumor activity of all chemotherapeutic agents.

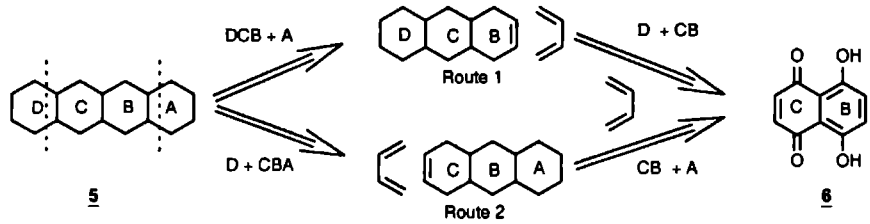
Figure S.1



Over the past 30 years an intensive research has been stimulated in order to find new and more active derivatives. Most of these methods were semi-synthetic. Only a few total syntheses of new adriamycin analogs have been described. The goal was to develop a method which allowed the highest possibility for variations in both structure and substituents.

The coupling of the aglycone 3 and the sugar daunosamine 4, as well as the synthesis of the sugar, were well described in the literature[1-6]. The work in this thesis is mainly focused towards the synthesis of the aglycone. Although the aglycone can be synthesized by several methods, the Diels-Alder approach is chosen. A priory several Diels-Alder strategies are possible 5 (Figure S.2) but, the methods described in this thesis all start from the commercially available naphthazarin 6.

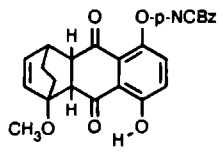
Figure S.2



In Chapter 2 a multigram synthesis of the aglycones (+/-)-daunomycinone 3 (R = H) and (+/-)-4-demethoxydaunomycinone via route 1 (A + BCD, Figure S.2) is described. When repeating the method of Kelly *et al.*[7] some serious problems were encountered.

In order to control the regioselectivity a mono *p*-nitrobenzyloxycarbonyl protected naphthazarin was used. To prevent the tedious oxidation of the *p*-nitrobenzyloxycarbonyl protected cycloadduct **7** (Figure S.3), the oxidized cycloadduct of naphthazarin **6** (Figure S.2) and 1-methoxy-1,3-cyclohexadiene is protected.

Figure S.3



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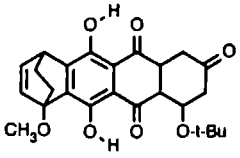

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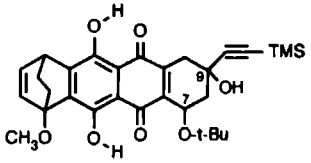
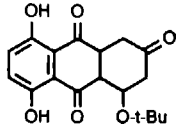


9 rac. mixt. of cis-7,9-OtBu,OH

In this way oxidized and protected cycloadduct is isolated on a multigram scale. Also the use of 1-*tert*-butoxy-3-trimethylsilyloxybuta-1,3-diene instead of 1,3-bis-trimethylsilyloxybuta-1,3-diene improves the solubility and stability of the hydrolyzed cycloadduct. In the subsequent Grignard reaction of **8** with 2-trimethylsilylthynyllithium the stereoselectivity is strongly controlled by the *tert*-butoxy group which results in the exclusive formation of the cis-7-*tert*-butoxy-9-hydroxy compound **9** (Figure S.3). The use of 2-trimethylsilylthynyllithium also reduces the number of ethynyl equivalents from 30 to 6. In this way both (+/-)-daunomycinone and (+/-)-4-demethoxydaunmycinone are synthesized on a gram scale with overall yields of 20% and 30%, respectively. The method as described in this Chapter has also been the basis for the synthesis of the new C_{13},C_{14} ethynyl analogs of daunomycin and 4-demethoxydaunomycin as described in Chapter 5.

In Chapter 3 the synthesis of (+/-)-daunomycinone is described following the ABC + D method (route 2, Figure S.2) which was originally developed by Krohn *et al.* This route is modified, improved and is used for the synthesis of new pyridine D ring analogs as described in Chapter 6. Diels-Alder reaction of naphthazarin **6** (Figure S.2) and 1-*tert*-butoxy-3-trimethylsilyloxy-buta-1,3-diene gives after oxidation the ABC fragment **10** (Figure S.4). Also here control of the stereoselectivity by the *tert*-butoxy group in the subsequent reaction with 2-trimethylsilylthynyl-lithium results in the exclusive formation of the cis-1-*tert*-butoxy-3-hydroxy compound **11a** (Figure S.4). In comparison to Krohn *et al.*[8] the tedious separation by preparative thin layer chromatography of the cis- and trans-1,3-dihydroxy compound **12** (Figure S.4) is prevented.

Figure S.4



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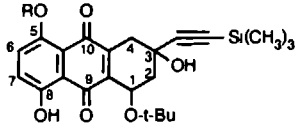
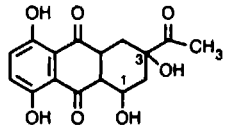


11a: R = H, **11b**: R=p-NO_2-C_6H_4-CH_2OCO

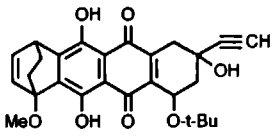
rac. mixt of cis-1,3-OtBu,OH



12

The subsequent Diels-Alder reaction of **11a** with 1-methoxy-1,3-cyclohexadiene gives as a main product the cycloadduct with the undesired regiochemistry. In order to prevent formation of the unwanted regioisomer compound **11a** is protected with *p*-nitrobenzyl chloroformate to give **11b** with a yield of 70%. Besides **11b**, small amounts of the other mono protected compound (8 position), the diprotected compound (5 and 8 positions) and **11a** are isolated. In the Diels-Alder reaction the regioselectivity is reversed, although, not completely (9 : 1). After oxidation (under the basic conditions of the oxidation the trimethylsilyl group is removed) and deprotection, the regioisomers are separated by column chromatography and compound **13** (Figure S.5) is converted into (+/-)-daunomycinone.

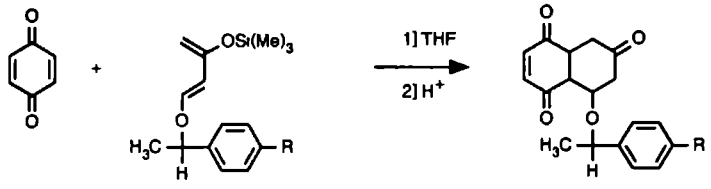
Figure S.5



13 rac mixt. of cis-7,9-OtBu,OH

In Chapter 4, an attempt towards the synthesis of optically active aglycones **3** (Figure S.1) is described. It is shown that it is possible to obtain the cycloadducts with a reasonable to high diastereomeric excess when using chiral 1-alkoxy-3-trimethylsilyloxybuta-1,3-dienes in the Diels-Alder reaction with quinones such as naphthazarin, juglone, naphthoquinone and benzoquinone. Especially in the reaction of benzoquinone **14** with 1-[1-(*p*-methoxyphenyl)-ethoxy]-3-trimethylsilyloxybuta-1,3-diene **15** a high d.e. is obtained, only one isomer **16** (Scheme S.1) is detected by ^{1}H-NMR spectroscopy. When the alkoxy group in **15** contained π or p electrons in the appropriate position the diastereomeric excess is larger than can be expected on account of the size of that particular group. This is explained using the perpendicular model of Siegel *et al*[9].

Scheme S.1



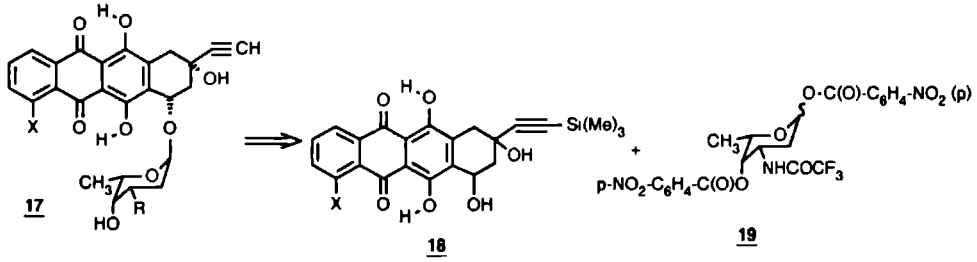
14 **15** R = OCH_3 **16** R = OCH_3

When using the S-(-)-diene **15** (R=H) derived from S-(-)-1-phenylethanol the absolute configuration (S) of the newly formed chiral center of compound **16** (R=H) is determined by X-ray analysis.

When applying the optical active diene 15 (R=H) in the Diels-Alder reaction with naphthazarin for the synthesis of the optically active aglycone, according to the method as described in Chapter 2, problems are encountered with the removal of the chiral alkoxy group. After Diels-Alder reaction, subsequent oxidation, introduction of the ethynyl group and transformation of that ethynyl group into an acetyl group an optically active compound is obtained. When the chiral alkoxy group is removed using trifluoroacetic acid, compound 12 is obtained (Figure S.4) but, this compound lost optical activity. Further investigations have to be focused on the use of an appropriate chiral alkoxy group which can be removed under mild acidic conditions to prevent epimerization of 12.

In Chapter 5, the synthesis of new C_{13},C_{14}-analogs of daunomycin 17 (X=OMe) and 4-demethoxydaunomycin 17 (X=H, Figure S.6) are described. These aglycones are prepared using the method as described in Chapter 2.

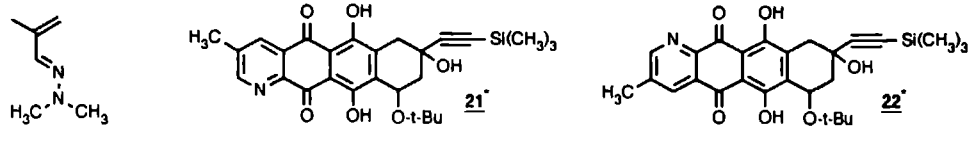
Figure S.6



According to the literature method of Terashima *et al.*[4] the aglycones 18 are coupled with the protected sugar daunosamine 19. After coupling of the sugar, separation of diastereomers is possible. The ethynyl analogs of daunomycin and 4-demethoxydaunomycin are prepared and tested on anti-tumor activity. The results of these test are reported in Chapter 8.

Chapter 6 describes the total synthesis of new pyridine D ring derivatives of daunomycin. The ABC ring fragments 11a/11b (Figure S.4), as synthesized in Chapter 3, are used in the Diels-Alder reaction with 1-(dimethylamine)-3-methyl-1-aza-buta-1,3-diene 20 to give the cycloadducts 21 and 22 after 1,4-elimination, oxidation and deprotection (Figure S.7).

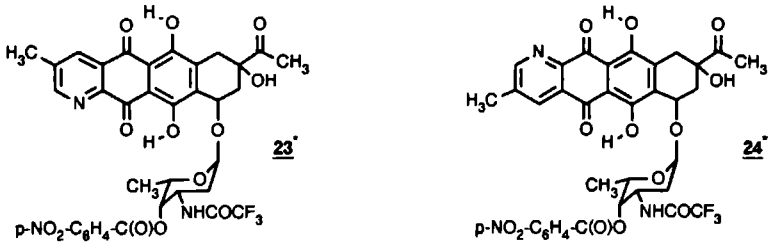
Figure S.7



The reactions are performed under high pressure (15 kbar). By using the *p*-nitrobenzyloxycarbonyl protecting group it is possible to influence the regioselectivity (ratio **21** : **22** is 1 : 2 for dienophile **11a**, the ratio is 7 : 3 for dienophile **11b**). At normal pressure conversion is much slower and decomposition of the ABC fragment is observed due to the elimination of water and *tert*-butyl alcohol.

The 2-trimethylsilylethynyl groups are hydrolyzed to the desired acetyl groups using HgO and diluted sulfuric acid. The racemic aglycones are coupled with the protected daunosamine (**19**, Figure S.6) to give the corresponding protected D-ring analogs of daunomycin **23** and **24** (Figure S.8).

Figure S.8



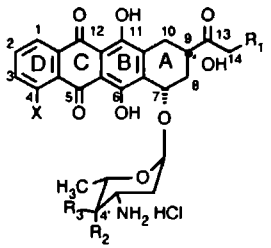
After deprotection, the HCl salts of the new D ring analogs are obtained and these are tested as racemic mixtures on their anti-tumor activity. The results of these tests are given in Chapter 8. For compound **24** it was possible to separate the diastereomers and both diastereomers are also tested on anti-tumor activity.

In Chapter 7 an overview is given of the biological activities of daunomycin and its derivatives. According to the literature five mechanisms contribute to the anti-tumor activity of this class of compounds. The mechanism of action is variable, complex and still partly understood. The most common side effect is the dose related cardiotoxicity.

Much effort has been devoted to the development of new analogs with a higher cytotoxic effect and a substantial lower cardiotoxicity. Most of these analogs were obtained by semi-synthesis. The anthracyclinones have 4 major moieties for modification (Figure S.9).

- Modification of the C_{13},C_{14} side chain.
- Modification of the BC rings.
- Modifications of the D ring.
- Modification of the sugar moiety.

Figure S.9

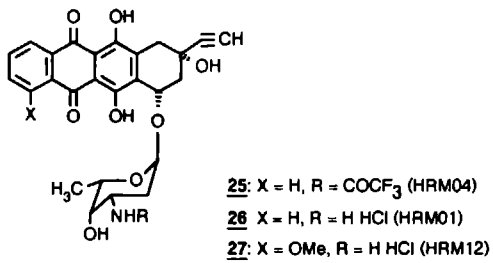


$X = OCH_3$	$R_1 = H$	$R_2 = OH$	$R_3 = H$	(daunomycin)
$X = OCH_3$	$R_1 = OH$	$R_2 = OH$	$R_3 = H$	(adriamycin)
$X = OH$	$R_1 = H$	$R_2 = OH$	$R_3 = H$	(carminomycin)
$X = H$	$R_1 = H$	$R_2 = OH$	$R_3 = H$	(idarubicin)
$X = OCH_3$	$R_1 = OH$	$R_2 = H$	$R_3 = OH$	(4'-epirubicin)

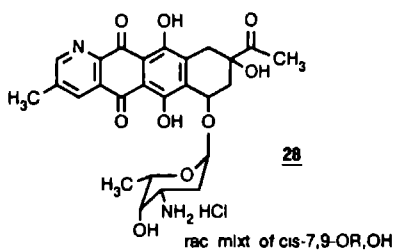
Although more than 2,000 derivatives have been synthesized and a part of them were tested on their biological activity, none of them ever could replace the parent compound in clinical use.

In Chapter 8 the test results of the new derivatives of daunomycin and their intermediates, as reported in the Chapters 5 and 6, are described. All new compounds are tested on their in vitro toxicity using five different human tumor cell lines. The test results show that the stereochemistry at position 7 and 9 is essential for the activity. From the C_{13},C_{14} ethynyl derivatives in general the HCl salts are the most active compounds and protection of the 3'-nitrogen group or the 4'-hydroxy group results in lower cytotoxic activity. Both compounds with a pyridine D ring show good promise. The HCl salt of 28 shows the highest activity (the compound is tested as pair of diastereomers). Four compounds are further investigated. The LD_{50}, in vivo L1210 cytotoxicity and in vitro cardiotoxicity are determined for compounds 25 - 28 (Figure S.10).

S.10



25: X = H, R = $COCF_3$ (HRM04)
26 X = H, R = H HCl (HRM01)
27: X = OMe, R = H HCl (HRM12)



28
rac mixt of cis-7,9-OR,OH

The results so far are promising for further testing of compound 28. In further testing, it is advisable to use only the 7S,9S diastereomer 28a. This compound shows a 8-10 times higher in vitro activity than the (1:1) mixture of diastereomers of 28.

References.

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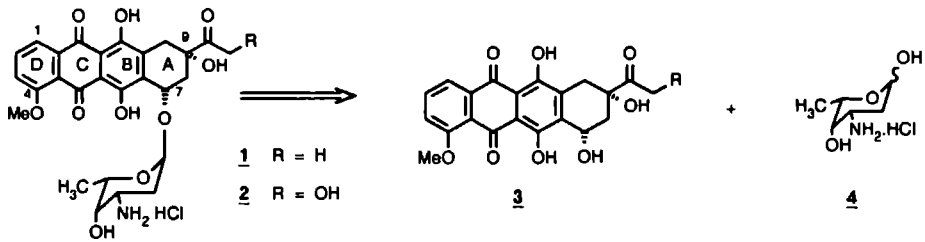
SAMENVATTING

In 1957 werd een rode kleurstof, die daunomycine 1 (Figuur S.1) werd genoemd, geïsoleerd uit een stam van microorganismen genaamd *Streptomyces peucetius*. Zes jaar later werd de antitumoractiviteit van deze verbinding aangetoond. In 1969 werd een tweede verbinding genaamd adriamycine 2, geïsoleerd uit een variant van de oorspronkelijk stam, *Streptomyces peucetius caesius*. Deze verbinding, die al sinds 1973 in de kliniek wordt toegepast, is heden ten dage nog steeds een van de meest gebruikte antitumorverbindingen met het grootste werkingsgebied van alle bestaande antitumorverbindingen.

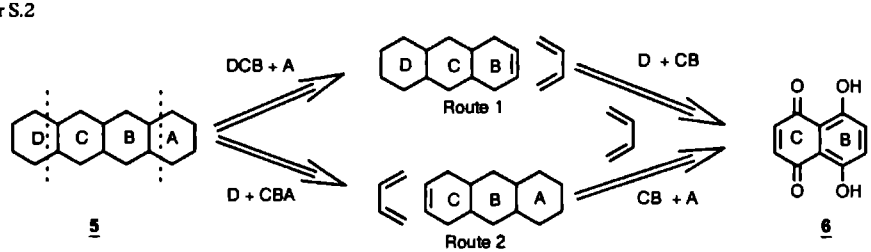
Figuur S.1



Gedurende de laatste 30 jaar is veel onderzoek gedaan om nieuwe en nog actievere derivaten te ontwikkelen. De meeste van deze methoden waren semi-synthetisch. Er zijn maar weinig voorbeelden beschreven waarin nieuwe derivaten zijn gemaakt via totaalsynthese. Doel van het onderzoek was om een zo flexibel mogelijke methode te ontwikkelen.

Zowel het koppelen van het aglycon 3 met de suiker daunosamine 4 als de synthese van de suiker zijn beschreven in de literatuur[1-6]. Daarom concentreet het onderzoek in dit proefschrift zich voornamelijk op de synthese van het aglycon. Hoewel het aglycon op verschillende manieren gemaakt kan worden is gekozen voor de Diels-Alder benadering. Op zich zijn meerdere Diels-Alder strategiën mogelijk 5 (Figuur S.2) maar dit proefschrift beschrijft enkele benaderingen die uitgaan van het commercieel verkrijgbare naftazarine 6.

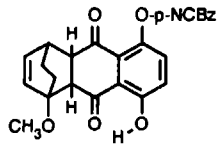
Figuur S.2



Hoofdstuk 2 beschrijft de synthese via route 1 (A + BCD, Figuur S.2) van de aglyconen daunmycinon 3 (R = H) en 4-demethoxydaunomycinon, op multigram schaal. Wannner daarbij

de route van Kelly *et al.*[7] wordt gevolgd, ontstaan er serieuze problemen. Kelly gebruikt naftazarine dat beschermd is met een *para*-nitrobenzyloxycarbonyl groep om de Diels-Alder reactie regioselectief te laten verlopen. Om de niet reproduceerbare oxydatie van het gevormde cycloadduct 7 (Figuur S.3) te vermijden is een methode ontwikkeld om het cycloadduct van naftazarine en 1-methoxy-1,3-cyclohexadieen te maken wat vervolgens regioselectief beschermd wordt met de door Kelly gebruikte beschermgroep.

Figuur S.3



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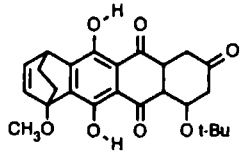

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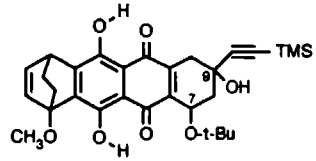
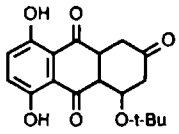


9 rac mengsel van cis-7,9-OtBu,OH

Op deze manier is mogelijk om het beschermde cycloadduct op multigram schaal te isoleren. Het gebruik van 1-*tert*-butoxy-3-trimethylsilyloxy-1,3-butadieen in plaats van 1,3-bistrimethylsilyloxy-1,3-butadieen levert een stabieler en beter oplosbaar cycloadduct op. In de daarop volgende Grignard-reactie met 2-trimethylsilylethynyllithium wordt de stereoselectiviteit bepaald door de *tert*-butoxy groep, met als gevolg dat alleen de cis-7-*tert*-butoxy-9-hydroxy verbinding wordt gevormd. Door gebruik te maken van 2-trimethylsilylethynyllithium kan het aantal ethynyl equivalenten teruggebracht worden van 30 naar 6. Op deze manier kan op een schaal van meerdere grammen zowel (+/-)-daunomycinon als (+/-)-4-demethoxydaunomycinon gesynthetiseerd worden met een overall opbrengst van respectievelijk 20% en 30%. De methode zoals beschreven in Hoofdstuk 2 is ook gebruikt voor de synthese van de C_{13},C_{14}-ethynyl derivaten van daunomycine en 4-demethoxydaunomycine zoals beschreven in Hoofdstuk 5.

In Hoofdstuk 3 is de synthese van (+/-)-daunomycine beschreven volgens de ABC + D methode (route 2, Figuur S.2) zoals oorspronkelijk ontwikkeld door Krohn *et al.*[8]. Deze methode is aangepast, verbetert en uiteindelijk gebruikt voor de synthese van nieuwe prydine ring-D derivaten zoals beschreven in Hoofdstuk 6. De Diels-Alder reactie van naftazarine 6 met 1-*tert*-butoxy-3-trimethylsilyloxy-1,3-butadieen resulteert na oxydatie in de vorming van het ABC fragment 10 (Figuur S.4). Ook hier zorgt de *tert*-butoxy groep voor de gewenste stereoselectiviteit in de Grignard-reactie met het 2-trimethylethynyllithium en wordt uitsluitend de gewenste cis-1-*tert*-butoxy-3-hydroxy verbinding 11a gevormd (Figuur S.4).

Figuur S 4



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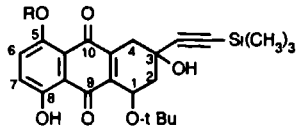
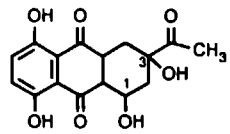


11a R = H, 11b R=p-NO_2-C_6H_4-CH_2OCO

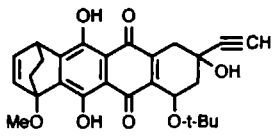
racemisch mengsel van cis-1,3-OtBu,OH



12

Diels-Alder reactie van verbinding **11a** en 1-methoxy-1,3-cyclohexadieen geeft als hoofdproduct het cycloadduct met de verkeerde regiochemie. Om te voorkomen dat deze verkeerde regio-isomeer ontstaat is verbinding **11a** regioselectief beschermd met de *p*-nitrobenzyloxy carbonylgroep. Verbinding **11b** wordt daarbij gevormd in een opbrengst van 70%. Naast deze verbinding worden ook de verbindingen gevormd met de beschermgroep op de 8 positie en de dubbel beschermde verbinding met de beschermgroep op de 5- en de 8-positie. Eveneens wordt een kleine hoeveelheid van de uitgangsstof teruggevonden. In de Diels-Alder reactie van **11b** blijkt dat de regioselectiviteit omgekeerd is, hoewel nog steeds een gedeelte (10%) van de verkeerde regio-isomeer wordt gevormd. Na oxydatie en ontscherming zijn de regio-isomeren gescheiden via kolomchromatografie. Door de basische omstandigheden van de oxydatie is de trimethylsilylgroep afgesplitst. Verbinding **13** (Figuur S.5) is via de in Hoofdstuk 2 beschreven methoden omgezet in (+/-)-daunomycinon.

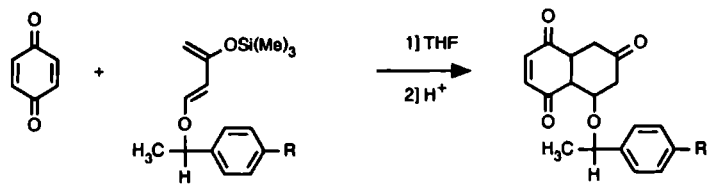
Figuur S.5



13 racemisch mengsel van cis-7,9-OtBu,OH

In Hoofdstuk 4 is een aanzet tot de synthese van de optisch actieve aglyconen **3** beschreven (Figuur S.1). Modelstudies hebben aangetoond dat het mogelijk is om Diels-Alder producten met redelijke tot hoge diastereomere overmaat te verkrijgen wanneer gebruikt gemaakt wordt van chirale 1-alkoxy-3-trimethylsilyloxy-1,3-butadienen in de Diels-Alder reactie met naftazarine, juglon, naftochinon en benzochinon. In de reactie van benzochinon **14** met 1-[1-(*p*-methoxyphenyl)ethoxy]-3-trimethylsilyloxybuta-1,3-diene **15** kan een zeer hoge diastereomere overmaat verkregen worden. Met behulp van ^{1}H-NMR spectroscopie kan slechts een diastereoisomeer waargenomen worden van verbinding **16** (Schema S.1). Wanneer de alkoxy groep in verbinding **15** op de juiste positie π- of p-electronen bevat, wordt een hogere diastereomere overmaat gevonden dan verwacht kan worden op grond van het verschil in grootte van de aryl en de methyl groep. Een verklaring hiervoor is gegeven met behulp van het 'perpendicular' model zoals beschreven door Siegel *et al.*[9].

Schema S.1



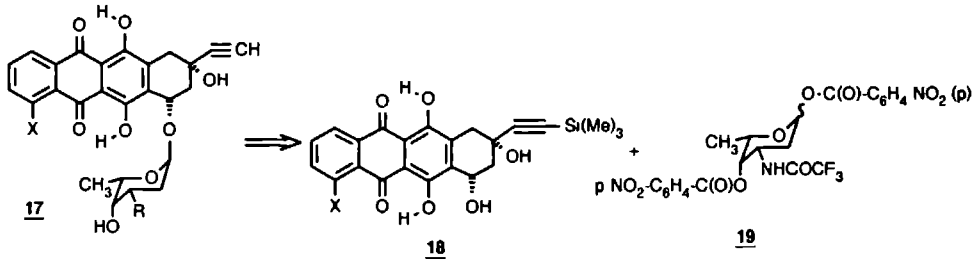
14 **15** R = OCH_3 **16** R = OCH_3

Door middel van X-ray kristallografie is de absolute configuratie (S) van het gevormde chirale centrum van verbinding **16** vastgesteld. Deze verbinding is verkregen via de Diels-Alder reactie van het S-(-)-dieen **15** (R = H), gemaakt vanuit S-(-)-1-phenylethanol, met benzochinon.

Bij gebruik van het optisch actieve dieen **15** (R=H) in de synthese van het optisch actieve aglycon via de Diels-Alder reactie met naftazarine zoals beschreven in Hoofdstuk 2, blijkt dat er problemen zijn bij het verwijderen van de chirale hulpgroep. Na Diels-Alder reactie en vervolgens oxydatie, invoering van de ethynyl groep en omzetting van die ethynyl groep in een acetyl groep wordt een optisch actieve verbinding verkregen. Bij het verwijderen van de hulpgroep met behulp van trifluorazijnzuur wordt wel verbinding **12** (Figuur S.4) verkregen, maar de optische activiteit is verdwenen. Verder onderzoek zal er op gericht moeten zijn om een geschikte chirale alkoxy groep te vinden die verwijdert kan worden onder mild zure omstandigheden zonder epimerisatie van verbinding **12**.

In Hoofdstuk 5 wordt de synthese van nieuwe C_{13},C_{14}-ethynyl derivaten van daunomycine **17** (X=OMe) en 4-demethoxydaunomycine **17** (X=H, Figuur S.6) beschreven. De aglyconen zijn gesynthetiseerd via de in Hoofdstuk 2 beschreven methode.

Figuur S 6



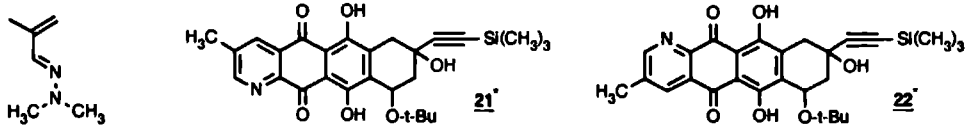
X = H, OMe

R = NH_2 HCl, $NHCOCF_3$

Volgens de literatuur methode van Terahima *et al.*[4] zijn deze aglyconen **18** gekoppeld met de beschermde daunosamine suiker **19**. Na koppeling met de suiker is het mogelijk om de diastereoisomeren te scheiden. De ethynyl derivaten van zowel daunomycine als 4-demethoxy-daunomycine zijn geïsoleerd en getest op hun antitumoractiviteit. De resultaten van deze tests staan beschreven in Hoofdstuk 8.

Hoofdstuk 6 beschrijft de totaal synthese van nieuwe pyridine ring-D derivaten van daunomycine. De ABC fragmenten **11a** en **11b** (Figuur S.4), waarvan de synthese is beschreven in Hoofdstuk 3, zijn gebruikt in de Diels-Alder reactie met 1-(dimethylamine)-3-methyl-1-aza--1,3-butadieen **20**. Na 1,4-eliminatie, oxydatie en ontscherming zijn de regio-isomere verbindingen **21** en **22** geïsoleerd (Figuur S.7).

Figuur S.7



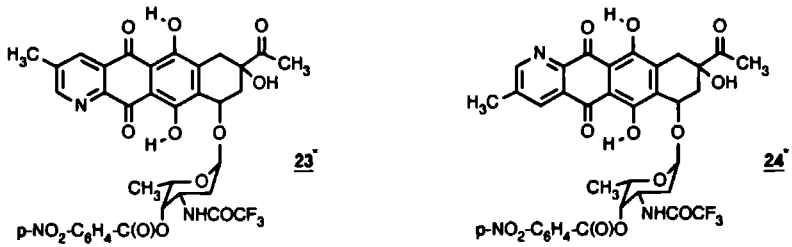
20 21* 22*

* = racemisch mengsel van cis-7,9-OtBu,OH

De reacties zijn uitgevoerd onder hoge druk (15 kbar). Door gebruik te maken van de *p*-nitrobenzyloxycarbonyl beschermgroep kan de regioselectiviteit van de reactie te worden beinvloed. Zonder beschermgroep (dieen 11a) is de verhouding tussen verbindingen 21 en 22 1 : 2, terwijl met beschermgroep (dieen 11b) de verhouding 7 : 3 is. Bij normale druk is de reactie veel langzamer en wordt ontleding van het ABC-fragment waargenomen via eliminatie van water en *tert*-butanol.

De 2-trimethylsilylethynyl groep is met behulp van HgO en verdund zwavelzuur omgezet in de acetyl groep. De racemische aglyconen zijn gekoppeld met de beschermde daunosamine-suiker 19 (Figuur S.6) en de beschermde pyridine ring-D derivaten van daunomycine 23 en 24 zijn geisoleerd (Figuur S.8).

Figuur S.8



23* 24*

* = racemisch mengsel van cis-7,9-OR,OH

Na ontscherming is het mogelijk om de HCl-zouten van de nieuwe ring-D derivaten te isoleren Deze zijn als mengsel van diastereoisomeren getest op hun antitumoractiviteit. De resultaten van deze tests zijn beschreven in Hoofdstuk 8. Voor verbinding 24 bleek het mogelijk om beide diastereoisomeren te scheiden en te testen op hun antitumoractiviteit.

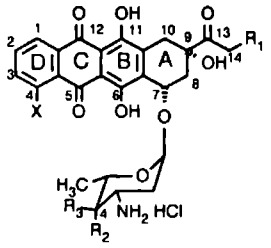
In Hoofdstuk 7 wordt een overzicht gegeven van de biologische activiteiten van daunomycine en derivaten. Volgens de literatuur zijn er vijf mechanismen die bijdragen aan de antitumorwerking van deze klasse van verbindingen. Het totale werkingsmechanisme is mede daardoor variabel, complex en nog steeds niet helemaal opgehelderd. De meest optredende nevenwerking van deze verbindingen is de aan de dosis gerelateerde cardiotoxiciteit.

Veel aandacht is in de literatuur besteed aan de ontwikkeling van nieuwe derivaten met een

hogere cytotoxiciteit en een aanzienlijk lagere cardiotoxiciteit. De meeste derivaten zijn gemaakt via semi-synthese. The anthracyclinonen kunnen op vier belangrijke plaatsen gewijzigd worden (Figuur S.9).

- Aanpasing van de C_{13},C_{14} zij keten.
- Aanpassing van het BC ringsysteem.
- Aanpassing van de ring-D.
- Aanpassing van de suiker eenheid.

Figuur S 9

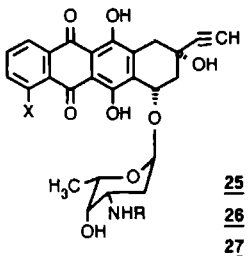


$X = OCH_3$	$R_1 = H$	$R_2 = OH$	$R_3 = H$	(daunomycin)
$X = OCH_3$	$R_1 = OH$	$R_2 = OH$	$R_3 = H$	(adriamycin)
$X = OH$	$R_1 = H$	$R_2 = OH$	$R_3 = H$	(carminomycin)
$X = H$	$R_1 = H$	$R_2 = OH$	$R_3 = H$	(idarubicin)
$X = OCH_3$	$R_1 = OH$	$R_2 = H$	$R_3 = OH$	(4'-epirubicin)

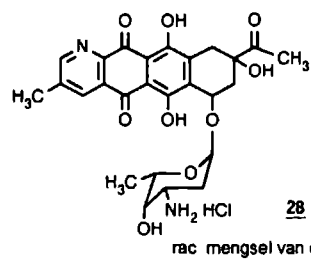
Hoewel tot nu toe meer dan 2000 nieuwe derivaten zijn gesynthetiseerd en een gedeelte daarvan is getest op biologische activiteit, is er nog geen derivaat gevonden dat adriamycine heeft vervangen in de klinische toepassing.

In Hoofdstuk 8 zijn de biologische testresultaten weergegeven van de nieuwe daunomycine derivaten en hun intermediairen zoals beschreven in de Hoofdstukken 5 en 6. Alle nieuwe verbindingen zijn getest op de in-vitro toxiciteit met behulp van vijf verschillende humane tumorcellijnen. De testresultaten tonen aan dat de stereochemie op de posities 7 en 9 essentieel zijn voor de activiteit. Van de C_{13},C_{14}-ethynyl derivaten zijn de HCl-zouten de meest actieve verbindingen. Bescherming van de 3'-amino of de 4'-hydroxy groep resulteerd in een lagere cytotoxiciteit. Beide verbindingen met een pyridine ring-D vertonen een hoge activiteit. De testresultaten van het HCl zout van verbinding 28 lijken veelbelovend, zeker gezien het feit dat deze verbinding werd getest als mengsel van diastereoisomeren. Vier verbindingen 25 - 28 zijn verder getest op hun LD_{50}, in-vivo cyctoxiciteit (L1210 test) en in-vitro carditoxiciteit.

Figuur S 10



25 X = H, R = $COCF_3$ (HRM04)
26 X = H, R = H HCl (HRM01)
27 X = OMe, R = H HCl (HRM12)



28
rac mengsel van cis-7,9-OR,OH

De resultaten voor verbinding 28 lijken tot nu toe erg goed. Wanneer verdere tests worden uitgevoerd is het aan te raden om alleen de 7S,9S diastereomeer van verbinding 28a te gebruiken. Deze verbinding blijkt een 8 tot 10 maal hogere in-vitro cytotoxiciteit te geven dan het (1:1) mengsel van diastereoisomeren van 28.

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CURRICULUM VITAE

Hans de Bie werd geboren op 23 juni 1961 te Waalwijk. In 1979 behaalde hij het diploma Atheneum B aan het Willem van Oranje college te Waalwijk en vervolgens werd een aanvang gemaakt met de studie scheikunde aan de Katholieke Universiteit te Nijmegen. Het kandidaatsexamen (S1) werd afgelegd in oktober 1983.
De doctoraalstudie omvatte als hoofdrichting Farmacochemie (Prof. Dr. J.M. van Rossum, onderwerp: Het aantonen en karakteriseren van de invloed van de hepato-entorale kringloop op farmacokinetische parameters met behulp van systeemdynamica). Daarnaast werd als bijvak (uitgebreid tot de omvang van een hoofdvak) Organische chemie gekozen (Prof. Dr. R.J.F. Nivard en Dr. J.W. Scheeren, onderwerp: Door hoge druk (10-12 kbar) gestimuleerde cycloaddities van furan aan electronenarme alkenen). Ter afronding van de doctoraal studie werd het caput college instrumentele methoden I met goed gevolg afgelegd. Na het afleggen van het doctoraal examen in december 1986 werd hij in maart 1987 aangesteld als toegevoegd onderzoeker aan de Katholieke Universiteit Nijmegen bij de vakgroep Organische Chemie, op een project gefinancieerd door Pharmachemie B.V. te Haarlem.
Het aldaar verrichte promotie onderzoek resulteerde onder de dagelijkse leiding van Dr. J.W. Scheeren en onder begeleiding Dr. D. de Vos van Pharmachemie B.V. in de totstandkoming van dit proefschrift.

Sinds 1 januari 1992 is hij werkzaam bij SPECS and BioSPECS B.V. te s' Gravenhage.